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PLANT BIOASSAYS FOR AN IN SITU MONITORING OF AIR NEAR AN INDUSTRIAL AREA AND A MUNICIPAL SOLID WASTE – ŽILINA (SLOVAKIA)

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Abstract. The process of a bioindication of genotoxic effects of complex mixtures on the environment using higher plants is very appropriate and effective. We present the results of an in situ indication of the genotoxic effects of polluted environment near Žilina city. For a more complex monitoring we used: the *Tradescantia* micronucleus (Trad-MCN) assay, the *Tradescantia* microspore test and an evaluation of the abortivity of the pollen grains of native plant species. We found significant differences in the frequency of the micronuclei when using the Trad-MCN test in local of Dubeň. The *Tradescantia* pollen abortivity test showed significant differences in the frequency of the abortive pollen grains between the exposed groups and the control group. By using native plant species in the pollen abortivity test we found significant differences in both of the two locations for the four following species during two consecutive years: *Artemisia vulgaris, Melilotus albus, Trifolium pratense, Typha latifolia*.

Keywords: genotoxicity, in situ, micronucleus, monitoring, pollen grains, plants, Slovakia, *Tradescantia*

1. Introduction

Many experimental studies have shown that, air, soil, or water in industrial areas contains various pollutants; most of them are combustion products (Sheu *et al*., 1997; Freeman and Tejada, 2002; Mitra *et al*., 2002; Erisman *et al*., 2003; Boughton and Horvath, 2004). These compounds do not act separately and the effects of chemicals in the complex mixtures on biological systems are still unclear.

There are different methods and monitoring strategies for a detection of environmental mutagenesis. In the late 1970s the US EPA (United States Environmental Protection Agency) Genetic Toxicology (Gene-Tox) program reviewed various bioassays. Plant test systems were also involved, because they are able to detect mutagens, clastogens and carcinogens (Ma, 1999). In the early 1980s a plant bioassay was incorporated in to the International Program on Chemical Safety (IPCS) under the World Health Organisation (WHO), the International Labour Organization (ILO), and the United Nations Environmental Program (UNEP) (Grant, 1994, 1999).

In our study we used the Trad-MCN test, which was recommended as an effective and reliable assay for an in situ monitoring (Ma, 1999; Grant, 1999; Ma *et al*., 1994, 1996). It is short-term, low-cost and well-suited bioassays, providing positive results at low levels of air contaminations in in situ condition (Ruiz *et al*., 1992; Monarca *et al*., 1999; Guimarães *et al*., 2000; Monarca *et al*., 2001; Isidori *et al*., 2003; Kim *et al*., 2003) and also shows a strong correlation with data obtained in the bacterial mutagenicity test (Monarca *et al*., 1999).

Apart from the Trad-MCN test a pollen grain abortivity test was used. This is one of the effective ways for an indication of the ecogenotoxicity using naturally occurring plant species. Pollen grains possess several characteristics, which make them suitable indicator and which arises from their haploid state, so all lethal mutations are manifested (Mulcahy, 1981). This test was as well carried out using pollen grains from exposed *Tradescantia* plants.

2. Material and Methods

2.1. Localities

Považský Chlmec is the locality in the vicinity of a municipal landfill site, which could be a source of potential mutagens from garbage. Other potential genotoxic substances could be formed during its degradation. The dumping site is located 2 km northwest from the industrial area of Žilina city. There are often spontaneous combustions in a landfill site, which commonly produce many air pollutants.

Dubeň locality is situated on a hill near the river Váh, in the vicinity (approximately 100–200 m) of the industrial complex Žilina city. The area is contaminated by pollutants such as SO_2, NO_x, NH_3, CO_2, CO (Table I) and various types of

TABLE I
Air pollution in Žilina for months July and August in year 2003

	MVM SO_2 (μg/m^3)	MAX SO_2 (μg/m^3)	MVM NO_x (μg/m^3)	MAX NO_x (μg/m^3)	MVM Dust (μg/m^3)	MAX Dust (μg/m^3)	MVM O_3 (μg/m^3)	MAX O_3 (μg/m^3)	MVM CO (μg/m^3)	MAX CO (μg/m^3)
July	11.36	60	20.17	97	22.19	136	65.15	170	263.6	1794
August	11.51	43	23.77	107	20.4	77	69.9	172	333.42	2726

Emission limits according to Appendix no. 1, Regulation No. 705/2000 of the Ministry of the Environment of the Slovak republic.
SO_2 – max 350 μg/m^3 per hour, average 125 μg/m^3 per 24 h.
NO_x – max 200 μg/m^3 per hour, average 40 μg/m^3 per year.
Dust – mean 50 μg/m^3 per 24 h, average 40 μg/m^3 per year.
CO – max 1000 μg/m^3 per 24 h, average 5 μg/m^3 per year.
O_3 – we have no emission limits for ozone.
MVM – mean value for month.
MAX – maximal value per hour.

organic compounds, which originate from mobile (intensive traffic in centre of city) and stationary sources (e.g. chemical factory for producing organic and inorganic chemicals and paper mill factory). A low dustiness, wind speeds of an average of 1.3 $m\,s^{-1}$ and up to a 60% occurrence of calm days per year characterise this area. These facts lead to the high level of air pollution at the ground level layer (MESR, 2003).

As a control locality for the pollen abortivity test carried out on native plant species a locality with a low level of polution in Záhorská nížina lowland, near village Moravský Jan was used. For the Trad-MCN we used the Department of Botany, Comenius University in Bratislava, Faculty of Natural Sciences.

2.2. Used plant bioassays

2.2.1. *Tradescantia Micronucleus (Trad-MCN) Assay*

Plants of *Tradescantia paludosa* E. Anders. et R.E. Woodson clone 03, with a standard cultivation, were exposed in pots to the observed areas for a longer period (11. 7.-30. 8. 2003 and 25. 7.-23. 8 2004). During this time, once a week, young flower buds were collected, which were immediately fixed in a mixture of ethanol (96%) and glacial acetic acid (3:1). After 24 h this mixture was replaced by a solution of 75% ethanol. Slide preparation and scoring of the tetrads were made according to the standardised protocols (Ma *et al.*, 1994). Controls were collected at the same time as the samples from the exposed local. The averages of the exposed and control samples were compared using the Student t-test and the difference between them was accepted at a 0.05 significance level.

2.2.2. *Pollen Grain Abortivity (Stainability) Test*

There were 8 commonly distributed plants species chosen from the surroundings of Žilina, which are appropriate according to the list of the suitable species (Murín, 1987; Mičieta and Murín, 1996). Young and closed flowers, before opening from a sufficient number of randomly selected individual plants (min. 10 individuals) were collected and fixed in a mixture of ethanol (96%) and glacial acetic acid (3:1). After 24 h, the fixing solution was replaced by 75% ethanol. Plant material was collected 3 times per year.

Flower buds were removed from the solution and the anthers were excised. The pollen grains were removed from each anther with a needle. For staining we used 1% aniline blue in lactophenol. After the stain penetration, the grains were covered by a cover glass and scored under a microscope. From each monitoring site, 3000 pollen grains were evaluated per sample.

Pollen grains were evaluated for their size, form and staining ability accordingly (Murín, 1987; Mičieta and Murín, 1996). Statistical significance of the differences between average frequency of the abortive pollen grains in control and impacted sites were evaluated using the Student t-test at a 0.05 significance level.

2.2.3. *Tradescantia Pollen Grain Abortivity (Stainability) Test*
From the same florets, which were used in the Trad-MCN test, young but mature flower buds were striped in one week intervals during the treatment. They were then used to evaluate the frequency of the abortive pollen in the same way as in test with the pollen grain of the native plant species. Statistical significance of the difference was evaluated by the Student t-test at a 0.05 significance level.

3. Result and Discussion

Tradescantia MCN test, based on the scoring of the micronuclei frequency in the early tetrads as a yield of the clastogenic attack to the meiotic chromosomes of the pollen mother cells is regarded as a very appropriate plant system (Ma *et al*., 1996).

In many studies, for the in situ monitoring of ambient air cuttings of plants were used, with the limited time for the treatment e.g. 5–7 h for a treatment (Ma *et al*., 1996), 8 h (Monarca *et al*., 2001), 9 h (Kim *et al*., 2003), and 24 h (Isidori *et al*., 2003; Monarca *et al*., 1999). The method similar to our, the exposure of the *Tradescantia* in the pots for longer time was used by Guimarães *et al*. (2000). The use of the plants in pots enabled us to monitor the level of the pollution for a longer time period. Tables II and III show the data obtained from the monitored localities using the Trad-MCN test. In two consecutive years after one week of an exposition there wasn't any statistically significant increase of micronucleus frequency observed. This fact confirms the theory, that for a better manifestation of pollution, a longer time for a treatment is needed. Guimarães *et al*. (2000), who used plants grown at the monitoring site and plants cultivated in pots, noted that the differences between the control and exposed groups were statistically significant. In our study we found a statistically significant increase in the frequency of micronuclei in the mother cells of the pollen grains only in the local of Dubeň (Figure 1), near a chemical factory and a paper mill factory. On the locality Považský Chlmec there weren't any statistical differences observed in the frequency of the micronuclei. This fact

TABLE II
Frequency of the micronuclei in *Tradescantia* placed in exposed localities during two months in the year 2003

	MCN ± S.D.						
Locality/Date	11.7.	22.7.	30.7.	11.8.	15.8.	23.8.	30.8.
Pov. Chlmec	2.0 ± 0.9	2.3 ± 0.8	2.4 ± 0.9	2.6 ± 1.3	2.3 ± 1.0	2.6 ± 0.8	2.5 ± 09
Dubeň	2.3 ± 1.0	2.9 ± 1.0*	3.2 ± 0.9*	3.5 ± 0.8*	3.3 ± 1.0*	2.9 ± 0.9*	3.1 ± 0.9*
Control	1.9 ± 0.9	2.0 ± 0.8	2.3 ± 0.7	2.5 ± 0.8	2.4 ± 0.7	2.1 ± 0.7	2.2 ± 0.7

MCN – frequency of micronuclei per 100 tetrads.
S.D. – standard deviation.
* $p \leq 0.05$.

TABLE III
Frequency of the micronuclei in *Tradescantia* placed in the exposed localities during two months in the year 2004

	Mean ± S.D.			
Locality/Date	25.7.	3.8.	13.8.	23.8.
Pov. Chlmec	2.2 ± 0.6	2.3 ± 0.7	2.3 ± 0.7	2.8 ± 0.6*
Dubeň	2.3 ± 0.8	3.7 ± 0.7*	3.9 ± 0.9*	3.6 ± 0.9*
Control	2.0 ± 0.7	1.9 ± 0.7	2.4 ± 0.6	2.0 ± 0.6

MCN – frequency of micronuclei per 100 tetrads.
S.D. – standard deviation.
* $p \leq 0.05$.

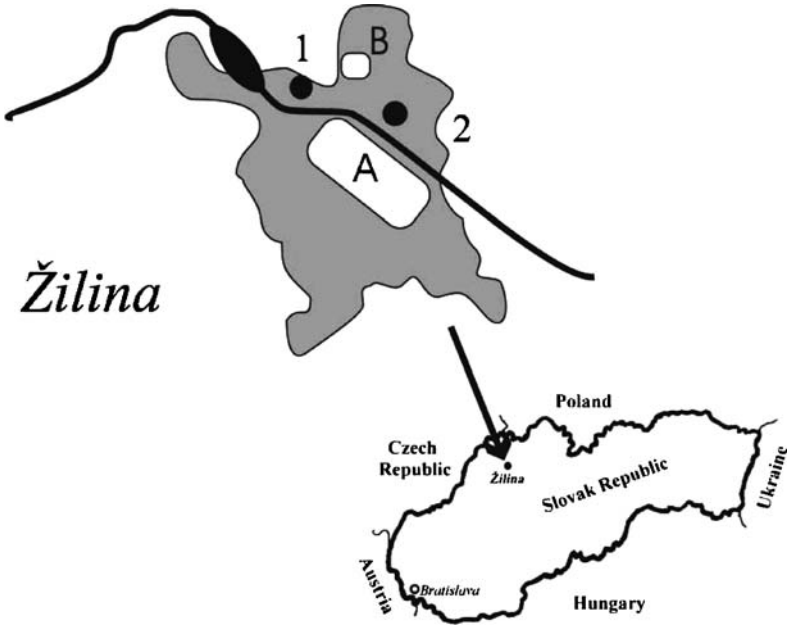

Figure 1. A schematic map illustrating the position of the Slovak Republic and the Žilina city. 1 – Považský Chlmec; 2 – Dubeň; A – industrial area; B – municipal landfill site.

was expected, because the local is situated far away from an industrial area, and the combustion in the landfill site during this season was not so frequent. In 2004 only four samples were collected because the plants had an insufficient number of inflorescens.

In the Tables IV and V, there are the results of the abortivity test on *Tradescantia*, which enabled us to evaluate the genotoxic effects of the pollutants present in the environment. In the year 2003 the cumulative effect of the pollutant is notice-

TABLE IV
Frequency of the pollen abortivity of *Tradescantia* in the year 2003

	Mean ± S.D.						
Locality/ Date	11.7.	22.7.	30.7.	11.8.	15.8.	23.8.	30.8.
Pov. Chlmec	3.1 ± 0.8	3.5 ± 1.0	4.1 ± 1.2	3.6 ± 1.1	4.5 ± 1.3*	5.7 ± 1.2*	6.2 ± 1.4*
Dubeň	3.3 ± 0.8	3.9 ± 1.2*	5.0 ± 1.3*	4.8 ± 1.5*	5.1 ± 1.4*	5.7 ± 1.1*	7.1 ± 1.4*
Control	3.4 ± 1.0	2.9 ± 0.9	3.2 ± 1.0	3.1 ± 0.9	3.1 ± 1.0	3.0 ± 0.9	3.3 ± 0.9

S.D. – standard deviation.
*$p \leq 0.05$.

TABLE V
Frequency of the pollen abortivity of *Tradescantia* in the year 2004

	Mean ± S.D.			
Locality/Date	25.7.	3.8.	13.8.	23.8.
Pov. Chlmec	3.8 ± 1.0	3.6 ± 1.1	4.1 ± 1.0	8.8 ± 3.5*
Dubeň	3.9 ± 0.9	4.1 ± 1.0	4.9 ± 1.1	5.1 ± 1.2*
Control	4.1 ± 1.0	3.8 ± 0.9	4.2 ± 1.2	3.9 ± 1.1

S.D. – standard deviation.
*$p \leq 0.05$.

able, when a longer time for a treatment caused an increase of the frequency of the abortion. In the year 2004, at the beginning the data varied, but at the end of the sampling period (the last sample) it showed an increase in the frequency of the abortive pollen at both of the monitored localities in comparison to the control. So the pollen abortivity test seems to be a better indicator of air pollution. The differences between the control and the exposed localities are higher than micronucleus frequency and we also found significant differences in the last three samples in the local of Považský Chlmec in 2003.

At present the method of an in situ monitoring by counting the abortive pollen grains (pollen viability measured as pollen stainability) of native plant species is well documented and it is possible to use it for various types of pollutions such as a refinery plant (Murín and Mičieta, 2000), aluminium plant and smelting plant (Mičieta and Murín, 1996–1998) as well as a nickel plant (Uhríková and Mičieta, 1997). Microspore analysis for a detection of the genotoxic risk is also used in areas with elevated levels of radioactivity (Murín *et al*., 1996; Kordyum and Sidorenko, 1997; Paradiž and Lovka, 1999) and with oil pollution of the environment such as in Kuwait (Murín and Malallah, 1994).

Regarding abortion of the pollen grains, significant increase of frequency of the abortive pollen was observed for both localities for the following species *Artemisia*

TABLE VI
Abortivity of the pollen grains in various plant species during the year 2003, values are means ± S.D.

	Mean ± S.D.		
	Pov. Chlmec	Dubeň	Control
Artemisia vulgaris L.	9.2 ± 2.0*	9.3 ± 3.6*	2.3 ± 0.2
Chelidonium majus L.	3.8 ± 1.6*	1.2 ± 1.0	2.2 ± 0.3
Daucus carota L.	4.3 ± 1.6	6.0 ± 2.3*	4.2 ± 0.8
Melilotus albus Medik.	5.1 ± 1.3*	7.2 ± 2.1*	1.1 ± 0.1
Ranunculus acris L.	1.3 ± 0.7	8.9 ± 3.9*	3.2 ± 0.4
Salix caprea L.	1.9 ± 1.1	0.9 ± 0.7	2.2 ± 0.3
Trifolium pratense L.	8.7 ± 1.6*	6.7 ± 4.3*	3.6 ± 0.6
Typha latifolia L.	2.5 ± 0.3*	3.1 ± 0.4*	0.8 ± 0.2

S.D. – standard deviation.
* $p \leq 0.05$.

vulgaris, Melilotus albus, Trifolium pratense, Typha latifolia and in 3 species (*Daucus carota, Chelidonium majus* and *Ranunculus acris*) at only one of the studied localities during the year 2003 (see Table VI). In the year 2004 statistically significant increase was observed at both localities for five species *Artemisia vulgaris, Chelidonium majus, Melilotus albus, Trifolium pratense, Ranunculus acris* (see Table VII). In this year (2004) we could not take samples from the species of *Typha*

TABLE VII
Abortivity of the pollen grains in selected plant species during the year 2004, values are means ± S.D.

	Mean ± S.D.		
	Pov. Chlmec	Dubeň	Control
Artemisia vulgaris L.	7.2 ± 2.2*	8.8 ± 1.8*	3.2 ± 0.9
Chelidonium majus L.	3.3 ± 1.1*	12.1 ± 5.8*	2.1 ± 0.1
Daucus carota L.	3.2 ± 1.2	4.1 ± 1.1*	3.6 ± 0.8
Melilotus albus Medik.	8.8 ± 2.5*	5.0 ± 2.2*	2.2 ± 1.1
Ranunculus acris L.	10 ± 4.2*	4.4 ± 1.0*	3.6 ± 0.7
Salix caprea L.	2.2 ± 0.7	2.9 ± 1.1*	2.1 ± 0.9
Trifolium pratense L.	5.2 ± 1.5*	4.1 ± 1.7*	1.6 ± 0.6
Typha latifolia L.		2.1 ± 0.7*	0.7 ± 0.6

S.D. – standard deviation.
* $p \leq 0.05$.

latifolia in the local of Pov. Chlmec because the municipal landfill site was being rebuilt. These species, which detect the presence of genotoxic factors in situ, we assumed to be suitable indicators, because their genetic materials react very sensitively to changes in the environment. In the observation of *Salix caprea* in the year 2003 we found lower frequency of the abortions of the pollen grains in the samples from the polluted locals in comparison to the control area. In this case we predict the tolerance of the population, especially, because of a long-term (cyclic or chronic) exposition is possible (Macnair, 1997). In many wild plants species, especially trees, the selection pressure of pollution has led to the natural evolutionary development of tolerant plant genotypes in response to wide range of various pollutants (Dickinson *et al*., 1991).

At present, the risk assessment of contaminated soil water and air is mainly based on chemical analyses, but this analytical approach doesn't allow us to detect the mixture toxicity. Use of a single bioassay will never provide a full picture of the quality of the environment. Only a test battery will provide enough knowledge to reduced the uncertainty and as well the details for an accurate assessment of the quality of the environment. There are many possibilities for the use of a plant test for an in situ monitoring; one of the various opportunities is using wild plant species, which grow in their natural habitat.

We used three plant bioassays in this study, which were used as screening tests for very dynamic changes in the environment for a monitoring of the ecogenotoxicity. This in situ approach and using more than one assay could be a useful tool for a routine monitoring of sites with industrial sources of pollution.

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