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In situ biomonitoring of the genotoxic effects of mixed industrial emissions using the Tradescantia micronucleus and pollen abortion tests with wild life plants: Demonstration of the efficacy of emission controls in an eastern European city

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Air pollution caused by industrial emissions induced micronuclei in Tradescantia and increased pollen abortion in wild plant species.

Abstract

Aim of the study was to monitor changes of genotoxic activity of urban air caused by an incinerator and a petrochemical plant in Tradescantia micronucleus (Trad-MCN) and pollen fertility assays with wild plants (*Chelidonium majus*, *Clematis vitalba*, *Cichorium intybus*, *Linaria vulgaris*, *Robinia pseudoacacia*). While in the first sampling period (1997–2000) significantly (on average 80%) more MN were found at the polluted site in comparison to controls from a rural area, no significant effects were observed during a later period (between 2003 and 2005). A similar pattern was observed in the pollen abortion assays in which the most pronounced effects were found in chicory and false acacia. The differences of the results obtained in the two periods can be explained by a substantial reduction of air pollution by use of new technologies. In particular the decrease of SO_2 emissions may account for the effects seen in the present study.

Keywords: *Tradescantia*; MCN assay; In situ monitoring; Bratislava; Pollen abortion

1. Introduction

Air pollution is considered by the World Health Organization (WHO) as one of the exposures that affect human health as it may lead to respiratory infections, cancer and to chronic respiratory and cardiovascular diseases (Yu, 2001). Although the role of air pollution in environmentally caused cancer is controversially debated, recent studies suggest that the incidence of lung cancer is elevated in urban environment (Dockery, 1993; Hemminki and Pershagen, 1994; Nyberg et al., 2000; Cheng, 2003; Bernstein et al., 2004).

Municipal waste incinerators are sources of organic and inorganic pollutants, which enter the environment via stack emissions. It was reported that the exhaust gas of incinerators contains chemicals such as polychlorinated dibenzo-*p*-dioxins, polychlorinated dibenzo-furans, nitroarenes, heavy metals, nitrosoamines and polycyclic aromatic hydrocarbons (Mowrer and Nordin, 1987; Kamiya and Ose, 1987a,b, 1988; Lisk, 1988; DeMarini et al., 1996). These pollutants have adverse effects on human health and in situ monitoring with bioindicators may provide useful information on potential health hazards (Isidori et al., 2003).

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Plant bioassays are cost and time effective and do not require specific equipment, excessive sample manipulation, and concentration procedures and have been used successfully for in situ exposure studies. For example, *Vicia faba* and *Arabidopsis thaliana* were used to monitor the genotoxic effects (sister chromatid exchanges and micronuclei) in air and soils polluted by industrial factories (Chroust et al., 1997), and single cell gel electrophoresis assays (SCGE) with ginkgo, pohtos and periwinkle were successfully employed in experiments to study air pollution caused by traffic emissions (Sriussadaporn et al., 2003). Furthermore, methods have been developed with bioindicators for the detection of specific air pollutants such as fluorides (Weinstein and Davison, 2003) and ozone (Bytnerowicz et al., 2002; Manning et al., 2002; Manning, 2003).

One of the most promising models for environmental monitoring is the Tradescantia micronucleus (Trad-MCN) assay, which has been employed successfully in a number of earlier investigations since the 1980s (see for example Ma et al., 1982; Monarca et al., 2001; Kim et al., 2003; Klumpp et al., 2006). The use of this test system for detection of genotoxins in environmental compartments (soil, air, water) is described in the reviews of Rodrigues et al. (1997) and Majer et al. (2005). Positive results were obtained for example in studies with traffic emissions and urban air (Monarca et al., 1999; Guimarães et al., 2000; Isidori et al., 2003; Carvalho-Oliveira et al., 2005; Klump et al., 2006), air pollutants released by the rubber industry (Monarca, 2001; Kim et al., 2003), gas stove emissions (Monarca et al., 1998), with air fresheners (Ma et al., 1982) and tobacco smoke (Ma and Harris, 1987). Also in studies concerning the release of gaseous toxins of incinerators, positive results were obtained (Ma et al., 1996; Fomin and Hafner, 1998) and it was shown that the test is able to detect effects of individual genotoxic carcinogens formed as combustion products (Ma et al., 1984; Rodrigues et al., 1997).

Another reliable bioassay for in situ monitoring are pollen abortive tests with wild plants as indicators (Murín, 1995; Mičieta and Murín, 1997). A description of the method and the criteria for the plant selection are given in the article of Murín (1995). This technique has been used for example for biomonitoring of metal contaminated soils (Uhríková and Mičieta, 1995; Mičieta and Murín, 1996), to detect the effects of environmental radionuclides (Kordyum and Sidorenko, 1997; Paradiž and Lovka, 1999) and to study the toxic effects of air pollution (Malallah et al., 1997; Mičieta and Kunová, 2000).

Plant abortion assays are highly sensitive since the target cells (microspores) are haploid and detect lethal mutations which affect the development of pollen. Another advantage of this experimental model is, that the indicator species are directly collected from the environment (Mičieta and Murín, 1997). One of the potential shortcomings of this approach is the possible adaptation of exposed populations to environmental toxins (for details see Mičieta and Murín, 1997).

The present work was aimed at monitoring the genotoxic effects of air pollution caused by gas emissions at a site located in the vicinity of the city incinerator and of a petrochemical plant in the city of Bratislava (Slovakia) with these bioassays. To draw conclusions on the persistence of the effects, the measurements were carried out over a period of 7 years. Before the start of the second sampling period, the incinerator was completely reconstructed, new filtration systems were installed and new production technologies came into use in the petrochemical plant.

2. Material and methods

2.1. Sampling site

Bratislava is the capital of the Slovak Republic; it has 453,000 inhabitants and covers a total area of 370 km^2. The monitoring site was located close to the city incinerator (distance 150 m) in the vicinity of a petrochemical plant (distance 200 m).

Fig. 1 shows the location of the sampling site, which was downwind from the two industries. Since all major traffic roads are more than 2 km away from the exposure site, a strong impact of car emissions on the out comings of the experiments can be excluded. In order to exclude that the effects are mainly due to release of toxins from particulate matter and not to gaseous emissions, the sampling was conducting in months with the lowest rainfall (July to September).

Table 1 summarizes the changes of relevant air quality parameters during the two periods. Measurements of the emissions of the incinerator show that the release of NO_x and CO_2 remained at relatively constant levels during the two periods while the amounts of particulate matter (PM), SO_2 and volatile organic compounds (VOC) decreased substantially. While the release of PM by the city incinerator was approximately 100 t/yr between 1997 and 2000, it was below 5 t/yr in 2004. Also at the petrochemical plant, which released similar amounts of PM, a decrease of 75% was recorded during the second sampling period (for details see: SAZP, 1997–2004: http://www.sazp.sk/slovak/periodika/sprava/index.html; NEIS: http://www.air.sk/neiscu/-main_gui.php; SLOVNAFT, 1997–2004: http://www.slovnaft.sk/showdoc.do?docid = 1167). Another important parameter that changed were the SO_2 emissions. During the first sampling period, the release by the incinerator was approximately 75 t/yr whereas it was only 2.2 t/yr in 2004. Likewise, the emissions of the petrochemical plant also decreased, being approximately 20,000 t/yr between 1997 and 1999 and in the second sampling period 50% lower (for details see web addresses given above).

The control location for Trad-MCN assays was a suburb with low pollution levels (garden of the Department of Botany of the Comenius University in Bratislava). For pollen abortion tests, plants were collected from a rural area with

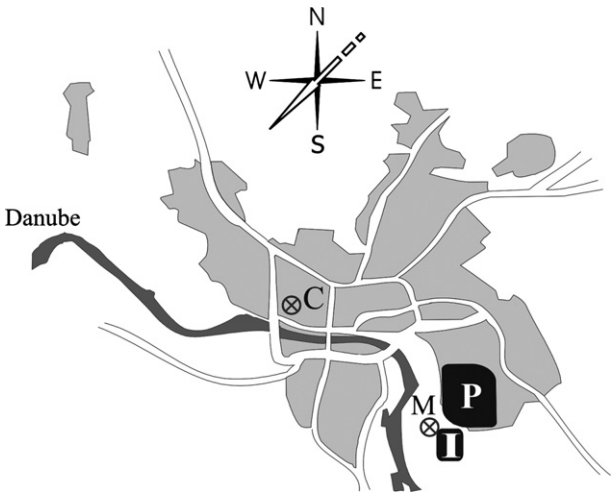

Fig. 1. Map of Bratislava city showing the monitoring sites: P, petrochemical plant; I, incinerator; M, exposure site of Tradescantia and collection area of wild plants; C, control site.

Table 1
Emissions of the two industries during the sampling periods[a]

	Emissions (t/year)					
	1997	1998	1999	2000	2003	2004
Petrochemical industry (Slovnaft)						
SO_2	21924	20310	20128	12894	12047	9672
NO_x	4461	4330	4364	4697	3715	3768
CO	703	698	693	763	567	722
PM[b]	1264	1207	1158	641	285	309
VOC[c]	–	7773	6439	6557	4567	4754
City incinerator						
SO_2	74	76	78	68	5	2
NO_x	112	114	118	103	156	177
CO	0	0	0	0	4	14
PM	107	109	112	98	1	1

[a] The table is based on data from the web site SAZP, 1997–2004; NEIS; SLOVNAFT, 1997–2004.
[b] PM, particulate mater.
[c] VOC, volatile organic compounds.

low pollution levels in the village Moravský Svätý Ján (Western Slovakia) at the same time as the samples from the exposed site.

2.2. Tradescantia micronucleus (Trad-MCN) assays

The *Tradescantia paludosa* clone #03 was obtained from J. Doležel (Institute of Experimental Botany, Olomouc, Czech Republic). Pots with at least 25 plants were transported to the sampling sites and exposed between July and September in the periods between 1997 and 2000, and between 2003 and 2005. The assays were carried out according to the protocol of Ma et al. (1994). The first samples were collected after an exposure period of ten days; subsequently young inflorescences were collected periodically each month. For each time point, 15 young flower buds were fixed in a mix of ethanol (96%) and glacial acetic acid (3:1). After 24 h, the inflorescences were transferred into 70% ethanol. The cells were stained with acetocarmine (Ma et al., 1994) and analyzed with a light microscope (400× magnification, Nikon YS2 Alphaphot). From each bud, one slide with cells in the early tetrad stage was prepared and per slide, 300 tetrads were evaluated for micronuclei (MCN) formation.

MCN are DNA-containing extracellular bodies surrounded by a plasma membrane, which are found as a consequence of chromosomal breakage (clastogenicity) or aneuploidy (Natarajan and Obe, 1982). They were scored according to the criteria defined by Tolbert et al. (1991, 1992); i.e. stained objects that were smaller than the nuclei and not connected to the nuclei were classified as MCN.

From each experimental point, five buds were scored (in total 1500 tetrads). Controls were collected at the same time as the samples from the exposed sites. Numbers presented in Table 2 indicate means ± standard deviations of MCN frequencies per 100 tetrads (i.e. percent of MCN).

To test for differences among the treatment groups within an experimental series ANOVA were used. If a significant p value ($p \leq 0.05$) was obtained, each treatment group and its corresponding negative control was tested for significance using the Dunnett's test for multiple comparisons.

2.3. Pollen grain abortion test with native species

Five common wild plants species, namely chicory (*Cichorium intybus* L.), old man's beard (*Clematis vitalba* L.), common toadflax (*Linaria vulgaris* Mill.), greater celandine (*Chelidonium majus* L.) and false acacia (*Robinia pseudoacacia* L.), were collected in the vicinity (20–30 m) of the Tradescantia exposure site during 1997–2000 and 2003–2005. All five species are known to be suitable for pollen abortion studies (Mičieta and Murín, 1996).

For each experimental point, flowers from at least 10 individuals were collected and 6000 pollen grains were evaluated (600 per plant). Flower buds were fixed in acetic acid/ethanol (3:1), after 24 h, the material was transferred into 70% ethanol. The buds were opened and the anthers were transferred to slides, stained with aniline blue (0.05%) in lactophenol (Darlington and La Cour, 1976). The pollen grains were released mechanically. After removal of the debris, the slides were covered and abortive grains evaluated under a light microscope (Nikon YS2 Alphaphot) under 400-fold magnification.

The main criteria for abortive pollen are size (grains which are significantly larger than the normal ones), altered forms (for example larger numbers of air bags or continual bag in the equatorial plane) and staining deficiencies (for details see Mičieta and Murín, 1996).

Statistical significance (comparison of the abortivity of pollen grains between control and polluted sites) was evaluated using Wilcoxon's signed rank test.

Table 2
Results of Tradescantia micronucleus (Trad-MCN) assays conducted in the two sampling periods (1997–2000 and 2003–2005)

Year	Location	Percent of MCN (±S.D.[a])		
		July	August	September
1997	Control	2.5 ± 0.5	2.5 ± 0.8	2.0 ± 0.9
	City	4.9 ± 0.8*	4.6 ± 1.2*	4.2 ± 1.3*
	IF[b]	1.9	1.8	2.1
1998	Control	1.7 ± 0.6	2.4 ± 0.6	1.7 ± 0.5
	City	4.9 ± 0.8*	4.3 ± 0.8*	4.3 ± 0.9*
	IF	2.8	1.8	2.5
1999	Control	2.6 ± 0.9	2.7 ± 0.7	2.3 ± 0.7
	City	4.7 ± 1.0*	4.1 ± 0.8*	3.7 ± 0.7*
	IF	1.8	1.5	1.6
2000	Control	2.6 ± 0.9	2.8 ± 1.1	2.8 ± 1.1
	City	4.7 ± 1.0*	4.1 ± 0.7*	4.2 ± 0.7*
	IF	1.8	1.5	1.5
2003	Control	2.1 ± 0.7	2.3 ± 0.8	2.6 ± 1.0
	City	2.8 ± 1.1	2.6 ± 0.8	3.2 ± 1.1
	IF	1.3	1.1	1.2
2004	Control	2.2 ± 0.8	1.9 ± 0.8	2.1 ± 0.8
	City	3.3 ± 0.8*	2.7 ± 1.3	3.1 ± 1.6
	IF	1.5	1.4	1.4
2005	Control	1.8 ± 1.5	2.1 ± 1.2	2.1 ± 1.1
	City	2.0 ± 1.6	2.5 ± 1.4	2.4 ± 0.9
	IF	1.1	1.2	1.1

*Statistical significance ($p \leq 0.05$).
[a] S.D., standard deviation.
[b] IF, induction factor: value recorded at the city site/control site.

3. Results

It can be seen in Table 2 and Fig. 2 that the numbers of MCN in Tradescantia were significantly higher than the background levels in the first period and remained relatively constant over the next 3 years. On average, the percentage of MCN was 80% higher than the background level (control site). In the second period, plants that were exposed close to the incinerator had still higher MCN numbers than those collected at the control site but the effects were substantially lower and not significant.

With few exceptions, significant increases of abortive pollen were seen in all species during the first period (Table 3, Fig. 3). The percentage of abortive grains in the control groups varied in the range between 0.7% and 6.3% and the lowest effects were consistently seen in *C. intybus*, whereas the highest values were found in *R. pseudoacacia*. The strongest increase at the polluted site was seen during the first period in *C. intybus* and *R. pseudoacacia*. In the former species, the number of abortive grains was in most cases twice as high as that recorded at the control site. In false acacia, the values varied over a broad range, the strongest decline of fertility being seen in 1999. During the second sampling period, no significant differences of the percentage of pollen abortion were found in general; however, in *Robinia* a significant effect was observed in the last year.

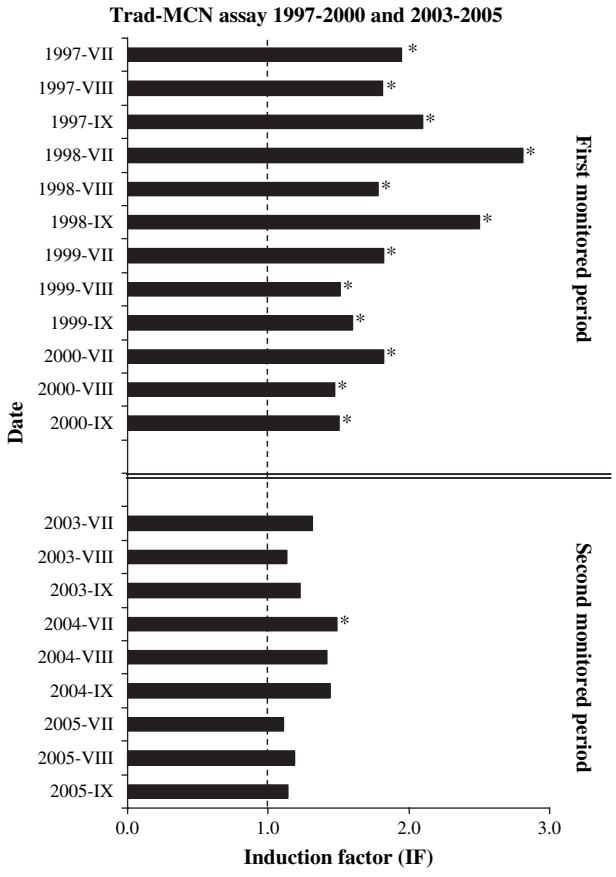

Fig. 2. The results of in situ monitoring with the Trad-MCN assay between 1997 and 2000, and between 2003 and 2005. The bars were calculated on the basis of the data shown in Table 1, and indicate the induction factors (percentage of MCN found at the polluted site/percentage of MCN found at the control site). *Statistical significance ($p \leq 0.05$).

4. Discussion

The results of the present study show that the contamination of urban air by a waste incinerator and a petrochemical plant caused strong genotoxic effects in Tradescantia during the first sampling period between 1997 and 2000 whereas no induction of MCN was seen in the second period (2003–2005; Table 2, Fig. 2). A similar difference was also found in the pollen abortion assays (Table 3, Fig. 3). This is an interesting observation as it indicates that the DNA-damaging effects detected in MCN tests with *Tradescantia* (chromosome breakage and aneuploidy) correlate with decreased fertility in wild plants. In this context, it is notable that also in another study in which the effects of different sources of urban air pollution in the Trad-MCN assay were compared with the fertility of three wild plant species, correlations of the effects were seen in the two models (Mišík et al., 2006).

As described in detail in the review of Rodrigues et al. (1997), it is well documented that the Trad-MCN assay detects the genotoxic effects of a air pollution (diesel exhaust fumes, gases such as SO_2 and NO_2, traffic, industrial and landfill emissions, tobacco smoke, etc.). These findings indicate that this bioassay can be used to compare the air quality in different areas and to conduct long term monitoring studies aimed at detecting changes in the pollutions levels (which was the purpose of present study).

The genotoxicity of emissions of incinerators has been investigated in Trad-MCN assays in two earlier studies and in both significant effects were observed. Ma et al. (1996) conducted in situ experiments at an incinerator in the United States (Illinois) and found a threefold increase over the background level. The second study was performed in Germany (Karlsruhe) (Fomin and Hafner, 1998) and also in this investigation a pronounced effect (20-fold induction over the background) was detected. In contrast to the present investigation, shorter exposure times (6–24 h) were used in these experiments and fewer individual experiments were conducted.

The results of the pollen abortion assays show that the background levels of the different indicator species vary over a broad range (Table 3, Fig. 3). At the control site, the lowest abortion levels were seen consistently in *C. intybus* (0.7–2.1%); the highest values were found in *L. vulgaris* (2.3–5.3%) and *R. pseudoacacia* (2.7–6.3%). Maximal induction of abortive pollen cells was found in *R. pseudoacacia* in 1999, but the results obtained with this plant varied strongly over the years and quite unexpectedly, a significant effect was observed in 2004 whereas with all other indicator plants consistently negative results were obtained at this sampling time. It is conceivable that the results seen in *R. pseudoacacia* are partly due to factors other than air pollution (e.g. contaminations of the ground water) and the strong variations distract from its use as a reliable indicator for air monitoring. The false

Table 3
Results of pollen abortion assays conducted in the two sampling periods (1997–2000 and 2003–2005)

Year	Location	Percent of abortive pollen grains (±S.D.[a])				
		Ch. majus	*C. vitalba*	*C. intybus*	*L. vulgaris*	*R. pseudoacacia*
1997	Control	2.6 ± 0.8	2.4 ± 0.9	2.1 ± 0.8	2.3 ± 0.9	4.2 ± 0.9
	City	1.6 ± 0.6	3.2 ± 1.0*	5.3 ± 1.5*	2.8 ± 1.2	5.8 ± 1.6*
	IF[b]	0.6	1.3	2.5	1.2	1.4
1998	Control	1.8 ± 0.7	2.9 ± 1.0	1.5 ± 0.6	2.6 ± 0.8	3.2 ± 0.9
	City	3.4 ± 1.0*	3.5 ± 0.9*	3.4 ± 0.9*	4.0 ± 0.7*	6.0 ± 1.4*
	IF	1.9	1.2	2.3	1.5	1.9
1999	Control	1.9 ± 1.0	2.1 ± 0.9	1.0 ± 0.5	3.2 ± 0.9	2.7 ± 1.1
	City	4.9 ± 1.8*	3.6 ± 1.0*	1.9 ± 0.7*	6.0 ± 1.5*	13.9 ± 3.6*
	IF	2.6	1.7	1.9	1.9	5.1
2000	Control	3.5 ± 1.0	–	1.1 ± 0.6	2.8 ± 1.2	3.5 ± 1.0
	City	6.1 ± 1.7*	–	3.2 ± 1.0*	5.2 ± 1.6*	4.0 ± 0.8
	IF	1.7	–	2.9	1.9	1.1
2003	Control	2.7 ± 1.2	3.6 ± 0.8	–	–	4.2 ± 0.9
	City	2.5 ± 0.8	4.1 ± 1.3	–	–	6.0 ± 1.5*
	IF	0.9	1.1	–	–	1.4
2004						
	Control	–	4.3 ± 1.5	1.1 ± 0.9	3.4 ± 1.7	4.8 ± 1.5
	City	–	4.5 ± 1.7	0.9 ± 0.7	3.9 ± 1.7	10.1 ± 4.2*
	IF	–	1.1	0.8	1.1	2.1
2005	Control	0.7 ± 0.7	3.0 ± 1.3	0.7 ± 0.9	5.3 ± 2.1	6.3 ± 2.3
	City	1.0 ± 0.8	3.4 ± 1.7	0.6 ± 0.9	6.2 ± 2.4	7.6 ± 3.6
	IF	1.4	1.1	0.9	1.2	1.3

*Statistical significance ($p \leq 0.05$).
[a] S.D., standard deviation.
[b] IF, induction factor: value recorded at the city site/control site.

acacia is the only tree used in present assays and has a much larger root system than the other indicator plants, which are all herbs. The results summarized in Table 1 show that *C. intybus* is clearly more sensitive than *Clematis vitalba*, *Linaria vulgaris* and *Chelidonium majus*.

The differences of the effects found in the two sampling periods, i.e. the clear positive responses during the first period and the lack of significant effects in the second which were apparent in both experimental models, can be explained by the implementation of an effective air filtration system in the incinerator and by use of new production technologies in the petrochemical plant including recuperation of gasoline vapors by a special recovery unit, establishment of electrostatic separators as well as construction of a polymer solid particle separator. The reduction of different pollution parameters is summarized in Table 1.

Emissions of incinerators and petrochemical plants contain a broad variety of compounds such as polyaromatic hydrocarbons, heavy metals, and nitroarenes, which may cause the mutagenic effects (DeMarini et al., 1996; Yoshino and Urano, 1998; Kim et al., 2005; He et al., 2006), therefore it is difficult to hypothesize which of them accounts for the effects seen in the present study.

One of the most important groups of genotoxic carcinogens found in gaseous emissions are polycyclic aromatic hydrocarbons, but these compounds give positive results in plant bioassays (including the Trad-MCN assay) only at high concentration, which are environmentally not relevant (Majer et al., 2005).

As mentioned above, one of the parameters which was strongly altered are the SO_2 emissions, and it is well documented that SO_2 causes induction of MCN in Tradescantia tetrads (Grant, 1993); furthermore, there is evidence that the gas causes also induction of stamen hair mutations and chromatid aberrations in pollen tubes of Tradescantia (Ma et al., 1973; Ma and Khan, 1976; Schairer et al., 1978). These effects are obviously not plant specific; recent findings provide evidence that the SO_2 also induces DNA damage in cultured human cells in vitro and in laboratory animals (Beckman and Nordenson, 1986; Pool et al., 1988). Also, in humans who were occupationally exposed to SO_2, increased chromosomal damage was found (Meng and Zhang, 1990; Meng et al., 1995; Yadav and Kaushik, 1996; Meng et al., 2004) and it was postulated that SO_2 exposure is related to increased lung cancer rates in workers (Lee et al., 2002).

5. Conclusions

Overall, the results of the present study show that both tests, i.e. the Tradescantia MCN assay as well as the pollen abortion tests with wild flowers, can be used to monitor alterations of pollution levels in urban air, and that both bioassays reflect the improvement in air parameters.

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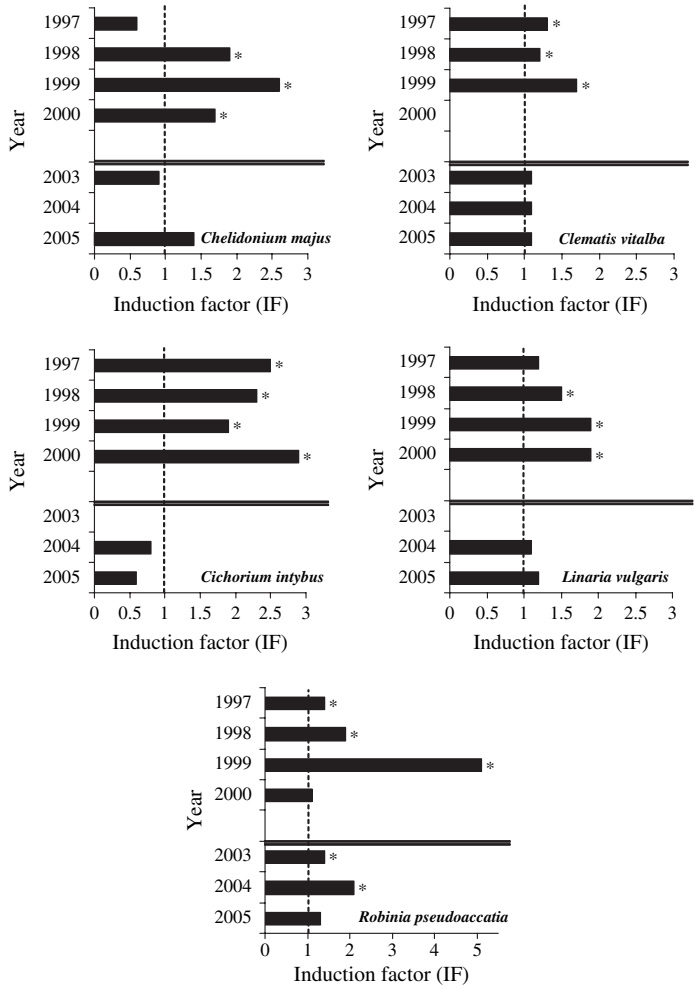

Fig. 3. Results of pollen abortion experiments with five plant wildlife species. The bars were calculated on the basis of the data presented in Table 3 and indicate induction factors (percentage of abortive pollen grains at the polluted site/percentage of abortive pollen grains at the control site). *Statistical significance ($p \leq 0.05$).

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