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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

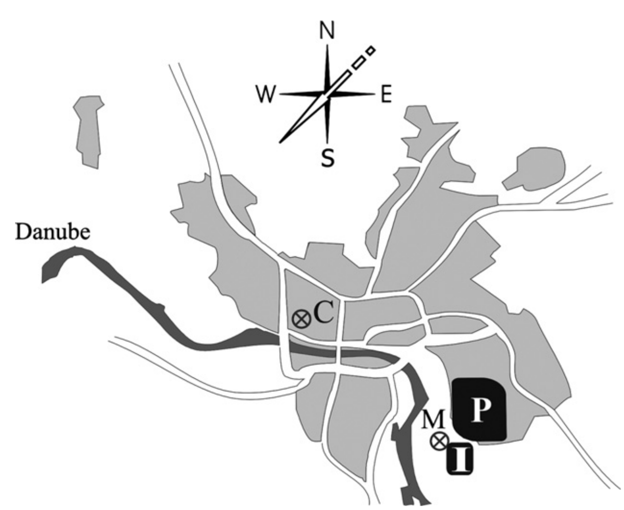

Fig. 1. Map of Bratislava city showing the monitoring sites: P, petrochemical plant; I, incinerator; M, exposure site of Tradescantia and collection area of wild plants; C, control site.