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Monitoring of volatile and non-volatile urban air genotoxins using bacteria, human cells and plants

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HIGHLIGHTS

- Urban air contains many mutagenic pollutants.
- Urban air in winter contains both volatile and non-volatile genotoxic substances.
- Air pollutants induce genetic damage in bacteria, human cells and plants.

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ABSTRACT

Urban air contains many mutagenic pollutants. This research aimed to investigate the presence of mutagens in the air by short-term mutagenicity tests using bacteria, human cells and plants.

Inflorescences of *Tradescantia* were exposed to air *in situ* for 6 h, once a month from January to May, to monitor volatile compounds and micronuclei frequency was computed. On the same days PM10 was collected continuously for 24 h. Half of each filter was extracted with organic solvents and studied by means of the Ames test, using *Salmonella typhimurium* TA98 and TA100 strains, and the comet assay on human leukocytes. A quarter of each filter was extracted with distilled water in which *Tradescantia* was exposed.

PM10 concentration was particularly high in the winter season (>50 μg/m^3). *In situ* exposure of inflorescences to urban air induced a significant increase in micronuclei frequency at all the sites considered, but only in January ($p < 0.01$). Aqueous extracts collected in January and February induced genotoxic effects in *Tradescantia* exposed in the laboratory ($p < 0.01$).

Ames test showed that organic extracts of winter urban air were able to induce genetic mutations in *S. typhimurium* TA98 strain (±S9), but not in TA100 strain, with a revertants/plate number nine times higher than the negative control. Comet assay showed that winter extracts were more toxic and genotoxic than spring extracts.

All the mutagenicity tests performed confirmed that urban air in North Italy in winter contains both volatile and non-volatile genotoxic substances able to induce genetic damage in bacteria, human cells and plants.

1. Introduction

Air pollution is a major environmental risk to health (WHO, 2005; Chen et al., 2008; ERS, 2010; Silva et al., 2013), causing acute respiratory infections, cancer, and chronic respiratory and cardiovascular diseases (Pope and Dockery, 2006; Schwarze et al., 2006; Kim et al., 2007; Chuang et al., 2007; Janssen et al., 2013; Raaschou-Nielsen et al., 2013; Shah et al., 2013). The main sources of air pollution in industrial countries are emissions from cars, domestic heating and factories, and urban air is a very complex and variable mixture of numerous classes and subclasses of contaminants, containing many different chemical species (WHO, 2005; EEA, 2012). The effects of exposure to such a mixture are difficult to determine because the different chemical species are not easily definable or measurable, and they can interact with additive, synergistic or antagonistic effects. Moreover, one or more

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chemicals can act according to different mechanisms and have multiple cellular targets (WHO, 2005, 2006; USEPA, 2008). The meta-analyses conducted on data obtained by the European Study of Cohorts for Air Pollution Effects, which used data from 17 cohort studies based in nine European countries, showed a statistically significant association between risk for lung cancer and PM_{10} (Raaschou-Nielsen et al., 2013). The International Agency for Research on Cancer has recently classified outdoor air pollution as carcinogenic to humans (Group 1) and concluded that there is sufficient evidence that exposure to outdoor air pollution causes lung cancer (Loomis et al., 2013). Particulate matter, a major component of outdoor air pollution, has been evaluated separately and has also been classified as carcinogenic to humans (Group 1).

The long-term pathological effects of chronic exposure to complex mixtures of air pollutants able to act at low concentrations should be taken into consideration. For a more complete evaluation of the health risk of human exposure, biological short-term assays were therefore performed to highlight mutagenic/carcinogenic activity of compounds present in urban particulate matter (PM) (Claxton et al., 2004; Claxton and Woodall, 2007; Vargas et al., 2011; Lemos et al., 2012; de Brito et al., 2013).

Short-term mutagenicity assays can directly detect the genetic effect of chemical and physical agents on the tested organisms, and they are able to assess the DNA damage resulting from exposure to mutagens. These tests are useful for defining the "environmental risk" deriving from both single chemicals and heterogeneous mixtures that act rapidly in reducing environmental genotoxic load (Buschini et al., 2001; Møller, 2005).

Numerous studies have demonstrated the genotoxicity of organic extracts of urban particulate matter (Monarca et al., 1999, 1997; Rodrigues et al., 1997; De Martinis et al., 1999; Cerná et al., 2000; Claxton et al., 2001; Zhao et al., 2002; Massolo et al., 2002; Vinitketkumnen et al., 2002; Abou Chakra et al., 2007; Bonetta et al., 2009; Coronas et al., 2009) and the connections between exposure to PM and the adverse effects on human health (WHO, 2006; Pope and Dockery, 2006; US EPA, 2008; Coronas et al., 2009; ERS, 2010). The fraction that has the greatest effects is fine particulates with a diameter of less than 10 μm (PM10), and especially less than 2.5 μm (PM2.5), because they adsorb many toxic and/or carcinogenic substances and can penetrate the lung alveoli, where they are retained at high rates (WHO, 2006; de Kok et al., 2006). PM10 consists of breathable particles to which several compounds, such as heavy metals, polycyclic aromatic hydrocarbons (PAHs), and some volatile compounds may adhere. Some of these compounds are able to induce genetic damage (Binková et al., 2003; Belpomme et al., 2007; Claxton and Woodall, 2007; Lewtas, 2007; Valavanidis et al., 2008). Research has confirmed the association between air pollution by PM10 and morbidity/mortality; PM10 is associated in particular with an increased incidence of lung cancer and cardiopulmonary mortality (Samet et al., 2000; Schwarze et al., 2006; Valavanidis et al., 2008; Janssen et al., 2013; Raaschou-Nielsen et al., 2013; Shah et al., 2013).

The Lombardy Region, in North Italy, is highly industrialised and has a high traffic density, where lung cancer rates are higher than the Italian average (Brescia LHA, 2012) and outdoor air quality standards for regulated air pollutants are exceeded quite frequently, with consequent endorsement of temporary emergency measures (Lombardy ARPA, 2011; EEA, 2012).

The *Tradescantia* micronucleus test has proved to be a suitable tool for monitoring the genotoxic potential of urban air pollution. Many studies have shown that plants are highly sensitive to atmospheric pollutants, such as sulphur dioxide, nitrogen oxide, ozone, formaldehyde, ammonia, and also to complex mixtures such as tobacco smoke and diesel exhaust (Rodrigues et al., 1997). *In situ* biomonitoring using higher plants may be useful for characterising air pollutants in areas even without any sophisticated instruments. Since urban air pollution comprises complex mixtures containing metals, organic compounds and secondary photochemical compounds, it is difficult to attribute increase in Trad-MCN frequency to a single, particular pollutant, but Guimarães et al. (2004), using a selective filtration technique, demonstrated that both particulate and gaseous fractions of air pollution are genotoxic. Significant associations were also detected between Trad-MCN frequency and adjusted mortality rate due to cardiovascular diseases and cancer (Mariani et al., 2009).

The Ames test is widely used for environmental studies (water sources, sediments and air, and for public water supply), it is recommended by international agencies for evaluating the mutagenic activity of different environmental samples and it is often mandatory in the initial screening for potential drugs to identify their possible carcinogenicity (OECD, 1997, 2010). The Ames test estimates genetic damage as point mutation (substitutions and deletion/insertion of basis), and due to its sensitivity in detecting and identifying mutagens commonly present in air, such as PAHs, nitro-compounds and aromatic amines, its use is continuously expanding (Claxton et al., 2004; Claxton and Woodall, 2007; Vargas et al., 2011).

The comet assay is used successfully both in monitoring air pollution mutagenicity and in detecting early biological effects on mutagen-exposed populations. It detects DNA double- and single-strand breaks, lesions in alkali-labile sites and incomplete repair of excision sites in different cell types, both *in vivo* and *in vitro*, supplying information about recent levels of exposure to genotoxic substances where part of the damage can still be repaired (Singh et al., 1988).

The aim of this study was to evaluate the presence of volatile and non-volatile mutagenic/genotoxic pollutants in urban air of Brescia, a town in the Lombardy Region, North Italy, by means of a battery of short-term mutagenicity bioassays on different types of cells: (a) the *Tradescantia*/micronuclei test (TRAD/MCN), applied both *in situ* and *in vitro* in the laboratory, to detect DNA damage through the formation of micronuclei in *Tradescantia* (clone #4430) pollen cells, derived from the exposure of the inflorescences to volatile and non-volatile pollutants; (b) the Ames test on TA98 and TA100 strains of *Salmonella typhimurium* to detect point mutations; c) the comet assay on human leukocytes to detect DNA strand breaks.

2. Materials and methods

In situ mutagenic activity of air pollutants was studied using the *Tradescantia*/micronuclei test. *In vitro* mutagenic/genotoxic activity of extracts of fine particulate matter was studied using the Ames test on bacteria to evaluate point mutations, comet assay on human leukocytes to evaluate DNA damage and *Tradescantia*/micronuclei test to evaluate chromosomal damage.

The research design, which is described below in detail, is set out in Fig. 1.

2.1. In situ Tradescantia/micronuclei (TRAD/MCN) test

The TRAD/MCN test was carried out using a hybrid of *T. hirsutiflora* and *T. subacaulis* (clone #4430) (Ma et al., 1994). Inflorescences of *Tradescantia* were exposed *in situ* for 6 h, from 10 a.m. to 4 p.m., to urban air at a height of 0.5 m above street level. The exposure was repeated every month, from January to May, at five different sites in the town, near the air monitoring stations of the Regional Agency for Environmental Protection (Lombardy ARPA, Italian acronym for Agenzia Regionale per la Protezione dell'Ambiente) that measure pollutants such as SO_2, NO_2, CO and PM10. The five sites were characterised by different traffic volumes: site 1 was an area with moderate car traffic; sites 2 and 4 were areas with high traffic; site 3 was an area with high traffic and near a waste incinerator; site 5 was a restricted traffic area.