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In situ monitoring of urban air in Córdoba, Argentina using the *Tradescantia*-micronucleus (Trad-MCN) bioassay

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Abstract

During the last decades, a significant deterioration of ambient air quality has been observed in Argentina. However, the availability of air pollution monitoring stations is still limited to only few cities. In this study, we investigated the genotoxicity of ambient levels of air pollution in Córdoba using the *Tradescantia* micronucleus assay. The experiment was performed from October, 2004 to April 2005. Pots with *Tradescantia pallida* were placed in three sites: Córdoba city center, characterized by important avenues with high traffic activity (cars, taxis, and public transport vehicles); the university campus, along a side road with heavy traffic of gasoline and diesel powered vehicles, buses and trucks; and a residential area, with no significant local sources of air pollution. Twenty young *T. pallida* inflorescences were collected from each sampling site in November, February and April. Micronuclei frequencies were determined in early tetrads of pollen mother cells and expressed as MCN/100 tetrads. Simultaneously, the environmental levels of total suspended particles (24 h mean) were determined for each site. A significant difference in micronuclei frequency was observed among sites ($p = 0.036$). Post-hoc analysis revealed that the residential area exhibited a lower micronuclei frequency than the university and city center areas. In conclusion, we found that the gradients of ambient air pollution of Córdoba are associated with changes in the spontaneous micronuclei frequency of *Tradescantia* pollen mother cells. These results indicate that *in situ* biomonitoring with higher plants may be useful for characterizing air pollution in areas without instrumental monitoring techniques, or for exploring the distribution of air contaminants at a microscale.

Keywords: Bioassay; Particulate matter; *In situ* biomonitoring; Clastogenicity; Argentina

1. Introduction

In Argentina, air pollution emissions are rapidly increasing due to population growth, industrial development and agricultural practices. There has consequently been a significant deterioration of ambient air quality during the last decades

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(Olcese and Toselli, 1997). Nonetheless, air monitoring networks were not established until the 1990s, and were then only implemented in a few cities. Therefore, the use of biomonitors represents an important contribution to air monitoring in Argentina, where measurements of particulate matter or other types of pollutants in large areas requires expensive technical equipment not yet available in the country.

The city of Córdoba is located in the center of Argentina and has a population of 1.4 million (2001 census). It is situated in a concave formation that reduces air circulation and causes frequent thermal inversions in autumn and winter. Due to the concentration of industrial and commercial activities in a relatively small area, it has suffered severe environmental degradation with higher than normal seasonal average values of NO_x, CO and PM_{10} (Olcese and Toselli, 1998). In actual fact, the peculiar arrangement of streets and buildings, weak ventilation, and slow traffic flow, means that polluting agents are unable to dissipate rapidly, particularly in the city center.

Increases in air pollution conceivably impact human health. Epidemiological studies have consistently reported significant associations between the exposure to urban levels of air pollution and acute and chronic health effects in many places around the globe, both in developed and developing countries (WHO, 2005). This association has been demonstrated in short-term time-series studies (Saldiva et al., 1995; Stieb et al., 2002; Zanobetti et al., 2003) and in long-term exposures (Abbey et al., 1999; Hoek et al., 2002; Pope et al., 2002). In the urban setting, areas subjected to high traffic density as well as industrial emissions are considered hot spots, where cumulative exposure to multiple pollutants enhances air toxicity. Thus, biological indicators that can put to evidence the effects of such complex mixtures are necessary to assess the risk of exposed populations.

The assessment of risk by air pollutants is generally based on concentrations detected by analytical chemistry and data on the toxicity and genotoxicity of single compounds, whereas the synergistic, additive or antagonistic effects of chemicals in complex air mixtures on biological systems are less known (Isidori et al., 2003). To assess health risks of pollutant mixtures, *in situ* monitoring of organisms may provide useful information on the species specific hazard that certain chemicals pose to the environment. Plant bioassays are best suited for addressing these clastogenic effects of 'real world' air pollution, with special emphasis on its soluble fraction, because they are more sensitive to environmental stresses than other currently available bioassay systems (Gopalan, 1999). Although the genotoxic effects detected by *Tradescantia* tests cannot be associated with mutagenesis or even carcinogenesis in humans, these bioassays are very useful tools for screening the mutagenic potential in the environment (Ma et al., 1994). *Tradescantia* can assess mutagenic effects by the formation of micronuclei in meiotic or mitotic cells and pink mutations in stamen hairs. The Trad-MCN bioassay has been used extensively for monitoring environmental genotoxicity, and it is particularly sensitive to chemical mutagens (Ma et al., 1994; Batalha et al., 1999; Monarca et al., 1999; Guimaraes et al., 2000). In this study, we used pollen mother cells of *Tradescantia pallida* Rose.-Hunt. cv. *purpurea* Boom, which is a popular ornamental plant widely grown in gardens, roadsides and streets of Córdoba.

Several biomonitoring studies using lichens and *Tillandsia* species have previously been carried out in Córdoba, revealing a significant accumulation of anthropogenic heavy metals (Carreras et al., 1998, Carreras and Pignata, 2001; Pignata et al., 2002). However, no information is available on the levels of genotoxic agents. In the present investigation we studied the genotoxicity of environmental pollution levels in three areas of Córdoba city with different levels of air pollution, using the *Tradescantia* micronucleus assay.

2. Materials and methods

2.1. Study sites

Córdoba city, capital of the province of Córdoba, is located at latitude 31°24′S and longitude 64°11′W, 440 m above sea level. It is characterized by a subhumid climate, with an average annual precipitation of 790 mm concentrated mainly in summer, a mean annual temperature of 17.4 °C and prevailing NE and SE winds. Its natural vegetation belongs to the Spinal Phytogeographical Province (Cabrera, 1976) which basically consists of low thorny woodlands.

Córdoba is one of the most polluted cities in the country. A variety of industrial plants are located in the suburban areas surrounding the city, including major automobile factories, auto-part industries, agro-industries and food processing companies.

However, it has previously been shown that these industries do not contribute significantly to air pollution, and that the main source of pollution comes from mobile sources (Stein and Toselli, 1996). Although the situation was critical, there were no available data on the quantity or quality of pollutants released into the atmosphere at the time the experiment was carried out. Moreover, these polluting substances can remain in the air for quite a long time during winter, due to the peculiar topology and anemological regime that characterizes this town. Additionally, the arrangement of streets and buildings, weak ventilation, and slow traffic, all contribute to the difficulty of dissipating polluting agents quickly, especially in the city center.

Three sites, with different environmental characteristics, were chosen in the urban area of Córdoba. One site corresponded to the city center (C) and is characterized by the presence of main avenues that concentrate traffic activity, cars, taxis, as well as public transportation vehicles, but no industries. This area is topographically depressed which, together with the high prevailing buildings, hinders pollution dispersion. Another sampling site was located in the university campus (UC), 3 km away from C, along a side road with heavy traffic of gasoline and diesel powered vehicles, buses and trucks. A third site was located in a residential district (R), 15 km away from the two other sites, surrounded by many green areas with abundant vegetation and hardly no traffic.

2.2. Total concentration of suspended particles

As there is no air monitoring network in Córdoba city, no air quality data was available for any of the sampling sites. The concentration of total suspended particles (TSP, 24 h mean) was determined gravimetrically for each site, using a medium volume sampler. Particulate material was collected on glass fiber filters, and accurately digested with a mixture of HNO_3, HCl and HFl. The digested product was then centrifuged, recovered in 10 ml Milli-Q water, and an internal standard of 100 μl Ge was added. Aliquots of 5 μl were taken from each solution and dried on an acrylic support. A calibration curve was determined using eight standard solutions with different concentrations of known elements. The particulate material collected on the filters and lichen thalli was quantified by synchrotron radiation spectroscopy (SR-TXRF). Samples were irradiated for 200 s with total reflection X-ray fluorescence (TXRF). A polychromatic beam of approximately 2 mm wide and 1 mm high was used for excitation, and a Si (Li) detector with a resolution of 165 eV at 5.9 keV was used for X-ray detection.

2.3. Experimental design

T. pallida plants were cultivated in pots (20 cm in diameter) using the same batch of commercial soil. The experiment was conducted from October 2004 to April 2005. During this period 10 pots were placed at each sampling site. In the city center plant samples were placed 10 m away from the main avenue and 3 m above ground level. In the university campus plant samples were placed in an interior garden of the Faculty of Engineering, at approximately 150 m from any roads, and in the residential area they were placed in the garden. In these two last sites, the samples were set on wood platforms 1 m above ground level. All pots were kept in the open air, without any shade, and watered twice a week. Every week, samples were closely inspected to keep them clean and free of pests. No pesticides were applied to or around the plants during the experiment.

2.4. Tradescantia-micronucleus assay

During the study period, 20 young inflorescences were collected from each sampling site during the months of November, February and April. The inflorescences were fixed overnight in a 1:3 glacial acetic acid–ethanol solution and then stored in 70% ethanol. The flowers were dissected and young anthers were squashed on a microslide in a solution of acetocarmine stain. Only preparations containing early tetrads were considered. Three-hundred tetrads were examined per slide at a magnification of 400X. Micronuclei frequencies were calculated by dividing the total number of micronuclei (MCN) by the total number of tetrads, and expressed as MCN/100 tetrads. Micronuclei were counted on coded slides and the code was only revealed after completing the entire experiment.

2.5. Statistical analysis

The results were evaluated with an analysis of variance and post-hoc contrasts were calculated with the Student–Neuman–Keuls method. When necessary, square root transformations were employed to homogenize variance among groups. The level of significance was set at 5%.

3. Results

Table 1 depicts the values of TSP and its elemental composition. Considerably high levels of ambient particulate matter were found in the C and UC areas, with TSP concentrations well above the annual limit of 150 μg/m^3 during several days. Similar values were observed in a previous study performed during June 1995–May 1996 (Olcese and Toselli, 1998). Higher concentrations of Cu, Zn, Sr and Pb were found at sites C and UC, probably due to their proximity to main roads. These elements have been reported to be related to traffic as they are constituents of leaded gasoline and of brake linings (Voutsa et al., 2002). The higher concentrations of Ca and Mn at sites C and UC can be related to crustal matter, and are usually the main components of PM_{10} (Roosli et al., 2001). The load of particulate matter in the R area was low, and so were the concentrations of all elements. It is worth noting that the concentrations of Pb, Ni and Zn greatly exceeded concentrations reported in other polluted areas (Voutsa et al., 2002). This could be due to the fact that the use of leaded gasoline in Argentina has decreased for over a decade. Viard et al. (2004) reported that in an area near a highway in France the amount of metal deposition was, in decreasing order, [Zn]>[Pb]>[Cd]. Therefore, it is possible that these metals are enriched in urban soils and hence become dispersed as particulate atmospheric matter. Regarding Zn, recent studies carried out by our research group have evidenced high atmospheric concentrations of this metal in urban areas of the province of Córdoba (Pignata et al. 2002; Wannaz and Pignata, 2006). High levels of Zn have also been reported in other areas where the dispersion of traffic emission contaminants is influenced by topographical characteristics and meteorological conditions (Viard et al., 2004).

Table 2 shows the micronuclei frequency of the three study sites. The values of micronuclei frequency were significantly different among the sites ($p = 0.036$). Post-hoc analysis revealed that the micronuclei frequency of the residential area is lower than the values obtained at the university and city center sites. Fig. 1 depicts the graphical relationship between micronuclei frequency and TSP levels for the three study sites, showing that micronuclei frequency increases as TSP levels became higher.

4. Discussion

In the present study, we demonstrated that *Tradescantia* plants kept in areas under the influence of traffic emissions evidence higher micronuclei frequencies than samples maintained in a region

Table 1
Mean values, range, standard error and elemental concentrations of total suspended particles (TSP) collected in the three sampling sites of Córdoba city

	C			UC			R			ANOVA
μg m^{-3}	Mean	Range	SE	Mean	Range	SE	Mean	Range	SE	*p* value
TSP	214.3 a	288.0	20.71	137.2 a	363.6	15.52	44.08 b	67.04	4.402	0.010
S	0.21	0.98	0.06	0.28	4.67	0.09	nd	nd	nd	ns
K	12.28	19.80	0.96	12.90	22.37	0.73	8.826	2.307	1.153	ns
Ca	5.79 a	12.12	0.58	3.95 b	8.80	0.26	3.615 b	4.537	2.268	0.005
Cr	0.02	0.44	0.02	0.02	0.56	0.01	0.000	0.000	0.000	ns
Mn	0.09 a	0.23	0.01	0.06 a	0.32	0.01	0.020 b	0.020	0.010	0.031
Fe	0.91	11.38	0.40	2.23	67.97	1.11	0.074	0.083	0.042	ns
Co	6.2×10^{-04}	6.4×10^{-03}	2.5×10^{-04}	2.2×10^{-03}	5.4×10^{-02}	1.1×10^{-03}	1.6×10^{-04}	3.2×10^{-04}	1.6×10^{-04}	ns
Ni	0.04	0.58	0.02	0.04	0.70	0.01	0.009	0.009	0.005	ns
Cu	0.09 a	0.12	0.01	0.03 b	0.16	0.00	0.013 b	0.013	0.006	0.000
Zn	18.60 a	25.82	1.27	14.38 ab	28.47	0.76	12.51 b	12.589	6.294	0.013
Br	6.5×10^{-03}	2.0×10^{-02}	9.4×10^{-04}	5.3×10^{-03}	2.4×10^{-02}	6.2×10^{-04}	3.7×10^{-03}	6.4×10^{-03}	3.2×10^{-03}	ns
Rb	8.9×10^{-03}	2.3×10^{-02}	1.2×10^{-03}	7.1×10^{-03}	4.3×10^{-02}	8.9×10^{-04}	5.4×10^{-03}	7.1×10^{-03}	3.5×10^{-03}	ns
Sr	2.5×10^{-02}a	6.9×10^{-02}	3.1×10^{-03}	1.6×10^{-02}ab	6.7×10^{-02}	1.8×10^{-03}	1.2×10^{-02}b	2.1×10^{-02}	1.1×10^{-02}	0.027
Pb	0.013	0.038	0.002	0.017	0.374	0.006	0.004	0.007	0.003	ns

Mean values on each vertical column followed by the same letter do not differ significantly. (nd: no data, ns: no significative).

Table 2
Descriptive statistics and analysis of variance result for the MCN frequencies measured in *T. pallida* plants in three different sampling sites of Córdoba city

Sites	*n*	Mean	Median	Minimum	Maximum	SD
C	16	4.236	3.495	1.33	10.67	2.671
UC	32	3.938	3.00	0.33	21.0	3.897
R	14	2.419	2.085	0.00	7.11	2.086
ANOVA (*p* value)		0.036				

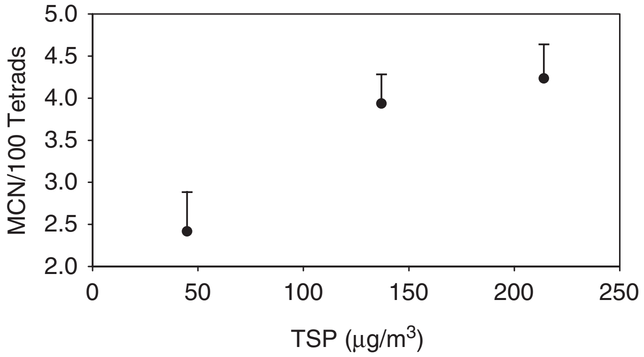

Fig. 1. Relationship between mean MCN frequencies (+standard error) measured in *Tradescantia* and total suspended particles (TSP) corresponding to three different sampling areas in Córdoba city.

devoid of high traffic load. Moreover, there is a positive dose–response relationship between micronuclei frequency and TSP, an estimator of air pollution, which is consistent with previous observations reporting that extracts of urban aerosol induced genotoxicity in animals, plants, mammalian cells and bacteria (Viras et al., 1991; Crebelli et al., 1995, Batalha et al., 1999). These results indicate that under field conditions, gradients of ambient levels of air pollution are associated with an indicator of DNA damage. This also confirms the results of Guimaraes et al. (2000), showing that gradients of air pollution commonly found in the urban scenario corresponded to changes in micronuclei frequency of the same plant system.

The present results are important for epidemiological studies focusing on the chronic effects of air pollution on human health. A cohort study of 500,000 people performed by Pope et al. (2002), showed that a 10 $\mu g/m^3$ increase of ambient levels of $PM_{2.5}$ was associated with an 8% increase in lung cancer rates. The fact that variations of ambient levels of particles are associated with significant changes in the spontaneous rate of micronuclei reinforces the concept that air pollution can cause malignancies in humans. Indeed, short-term assays in ambient conditions may help to predict the risk of genotoxicity of urban atmospheres before actual risk in humans is computed. The estimation of risk attributed to low levels of air pollution in the pathogenesis of human cancer, using only epidemiological tools, generally demands long-term studies and the enrollment of a large number of individuals. This scenario has its disadvantages. Firstly, when the association is clearly established a large number of people have already developed the disease. Secondly, these studies are usually not feasible in developing economies due to the cost of large cohorts and the need to have good air monitoring data. Thus, even though mutagenesis in plants and the risk for human cancer cannot be equated, these plant bioassays can be very useful as screening instruments for assessing human risk. Studies such as the present one, that reveal a relationship between genetic damage in *Tradescantia* and ambient concentrations of a known human carcinogenic condition, encourage the use of plant bioassays for air pollution monitoring.

Due to the flexibility, low cost and efficiency of the plant system used in this study, another important issue of environmental epidemiology in urban areas was addressed—pollution hot spots. Hot spots are certain areas in a city where pollution is significantly higher because of low dispersion caused by street canyons or high traffic density. It is extremely possible that the inhabitants of areas with these conditions have a higher risk of developing adverse health effects. In the present study we demonstrated that low air convection in the center of Córdoba city, and the proximity of a highway to the University, affected the rate of *Tradescantia* micronuclei frequency, indicating the possibility of identifying areas with higher biological adverse effects. These results show that differences in air pollution levels can be determined using the biological response of a higher plant system as a pollution estimator.

Considering that urban air pollution is comprised by a complex mixture containing metals, organic compounds and secondary photochemical compounds, it is difficult to ascribe the increase in micronuclei frequency observed in our study to a particular agent. By selective filtration, Guimaraes et al. (2004) demonstrated that both particulate and gaseous fractions of air pollution are genotoxic. Indeed, the degree of correlation between gaseous and particulate fractions is noticeably high in urban settings (Saldiva et al., 1995). In this scenario, it is preferable to consider our TSP measurements as proxy variables of total air pollution load, rather than attributing them only to the particles responsible of the genotoxic effects in our study.

In conclusion, we found that the gradients of ambient levels of air pollution within the city of Córdoba are associated with spontaneous changes in micronuclei frequency of *Tradescantia* pollen mother cells. These results indicate that *in situ* biomonitoring using higher plants may be useful for characterizing air pollution in areas without instrumental monitoring techniques, or for exploring the distribution of air contaminants at a microscale.

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References

Abbey, D.E., Nishino, N., McDonnell, W.F., Burchette, R.J., Knutsen, S.F., Lawrence Beeson, W., Yang, J.X., 1999. Long-term inhalable particles and other air pollutants related to mortality in nonsmokers. American Journal of Respiratory and Critical Care Medicine 159, 373–382.

Batalha, X.R.F., Guimaraes, E.T., Lobo, D.J.A., Lichtenfels, A.J.F.C., Deur, T., Carvalho, H.A., Alves, E.S., Domingos, M., Rodrigues, G.S., 1999. Exploring the clastogenic effects of air pollution in São Paulo (Brazil) using the Tradescantia micronuclei assay. Mutation Research 426, 229–232.

Cabrera, A.L., 1976. Regiones fitogeográficas argentinas. Enciclopedia Argentina de Agricultura y Ganadería, 2 ed. ACME, Buenos Aires, 85pp.

Carreras, H.A., Pignata, M.L., 2001. Comparison among air pollutants, meteorological conditions and some chemical parameters in the transplanted lichen *Usnea amblyoclada*. Environmental Pollution 111, 45–52.

Carreras, H.A., Gudiño, G.L., Pignata, M.L., 1998. Comparative biomonitoring of atmospheric quality in five zones of Córdoba city (Argentina) employing the transplanted lichen *Usnea* sp. Environmental Pollution 103, 317–325.

Crebelli, R., Fuselli, S., Baldassarri, L.T., Ziemacki, G., Carere, A., Benigni, R., 1995. Genotoxicity of urban air particulate matter: correlations between mutagenicity data, airborne micropollutants and meteorological parameters. International Journal of Environmental Health 5, 19–34.

Gopalan, H.N.B., 1999. Ecosystem health and human well being: the mission of the international program on plant bioassays. Mutation Research 426, 99–102.

Guimaraes, E.T., Domingos, M., Alves, E.S., Caldini Jr., N., Lobo, D.J.A., Lichtenfels, A.J.F.C., Saldiva, P.H.N., 2000. Detection of the genotoxicity of air pollutants in and around the city of São Paulo (Brazil) with the Tradescantia-micronucleus (Trad-MCN) assay. Environmental and Experimental Botany 44, 1–8.

Guimaraes, E.T., Macchione, M., Lobo, D.J., Domingos, M., Saldiva, P.H., 2004. Evaluation of the mutagenic potential of urban air pollution in São Paulo, Southeastern Brazil, using the *Tradescantia* stamen-hair assay. Environmental Toxicology 19, 578–584.

Hoek, G., Brunekreef, B., Goldbohm, S., Fischer, P., Van den Brandt, P.A., 2002. Association between mortality and indicators of traffic-related air pollution in the Netherlands: a cohort study. Lancet 360, 1203–1209.

Isidori, M., Ferrara, M., Lavorgna, M., Nardelli, A., Parrella, A., 2003. In situ monitoring of urban air in Southern Italy with the Tradescantia micronucleus bioassay and semi-permeable membrane devices (SPMDs). Chemosphere 52, 121–126.

Ma, T.H., Cabrera, G.L., Chen, R., Gill, B.S., Sandhu, S.S., Vandenberg, A.L., Salamone, M.F., 1994. Tradescantia micronucleus bioassay. Mutation Research 310, 221–230.

Monarca, S., Feretti, D., Zanardini, A., Falistocco, E., Nardi, G., 1999. Monitoring of mutagens in urban air samples. Mutation Research 426, 189–192.

Olcese, L.E., Toselli, B.M., 1997. Effects of meteorology and land use on ambient measurements of primary pollutants in Córdoba city, Argentina. Meteorology and Atmospheric Physics 62, 241–248.

Olcese, L.E., Toselli, B.M., 1998. Statistical analysis of PM10 measurements in Córdoba City, Argentina. Meteorology and Atmospheric Physics 66, 123–130.

Pignata, M.L., Gudiño, G.L., Wannaz, E.D., Plá, R.R., González, C.M., Carreras, H., Orellana, L., 2002. Heavy metals distribution and atmospheric quality in Argentina employing *Tillandsia capillaris* as biomonitor. Environmental Pollution 120, 59–68.

Pope 3rd, C.A., Burnett, R.T., Thun, M.J., Calle, E.E., Krewski, D., Ito, K., Thurston, G.D., 2002. Lung cancer, cardiopulmonary mortality, and long-term exposure to fine particulate air pollution. Journal of the American Medical Association 287, 1132–1141.

Roosli, M., Theis, G., Kunzli, N., Stachelin, J., Mathys, P., Oglesby, L., Comenzid, M., Braun-Fahrlanden, Ch., 2001. Temporal and spatial variation of chemical composition of PM_{10} at urban and rural sites in the Basel area, Switzerland. Atmospheric Environment 35, 3701–3713.

Saldiva, P.H., Pope 3rd, C.A., Schwartz, J., Dockery, D.W., Lichtenfels, A.J., Salge, J.M., Barone, I., Bohm, G.M., 1995. Air pollution and mortality in elderly people: a time-series study in São Paulo, Brazil. Archives of Environmental Health 50, 159–163.

Stein, A.F., Toselli, B.M., 1996. Street level air pollution in Córdoba city, Argentina. Atmospheric Environment 30, 3941–3945.

Stieb, D.M., Judek, S., Burnett, R.T., 2002. Meta-analysis of time-series studies of air pollution and mortality: effects of gases and particles and the influence of cause of death, age, and season. Journal of Air & Waste Management Association 52, 470–484.

Viard, B., Pihan, F., Promeyrat, S., Pihan, J.C., 2004. Integrated assessment of heavy metal (Pb, Zn, Cd) highway pollution: bioaccumulation in soil, Graminaceae and land snails. Chemosphere 55, 1349–1359.

Viras, L.G., Siskos, P.A., Samara, C., Kouimtzis, T., Athanasiou, K., Vavatzandis, A., 1991. Polycyclic aromatic hydrocarbons and mutagens in ambient air particles sampled in Thessaloniki, Greece. Atmospheric Environment 10, 999–1007.

Voutsa, D., Samara, C., Kouimtz, Th., Ochsenkün, K., 2002. Elemental composition of airborne particulate matter in the multi-impacted urban area of Thessaloniki, Greece. Atmospheric Environment 36, 4453–4462.

Wannaz, E., Pignata, M.L., 2006. Calibration of four species of Tillandsia as air pollution biomonitors. Journal of Atmospheric Chemistry 53, 185–209.

World Health Organization (WHO), 2005. The effects of air pollution on children's health and development: a review of the evidence. WHO Regional Office for Europe, 185pp., downloaded from ⟨ ⟩http://www.euro.who.int/air⟩.

Zanobetti, A., Schwartz, J., Samoli, E., Gryparis, A., Touloumi, G., Peacock, J., Anderson, R.H., Le Tertre, A., Bobros, J., Celko, M., Goren, A., Forsberg, B., Michelozzi, P., Rabczenko, D., Hoyos, S.P., Wichmann, H.E., Katsouyanni, K., 2003. The temporal pattern of respiratory and heart disease mortality in response to air pollution. Environmental Health Perspectives 111, 1188–1193.