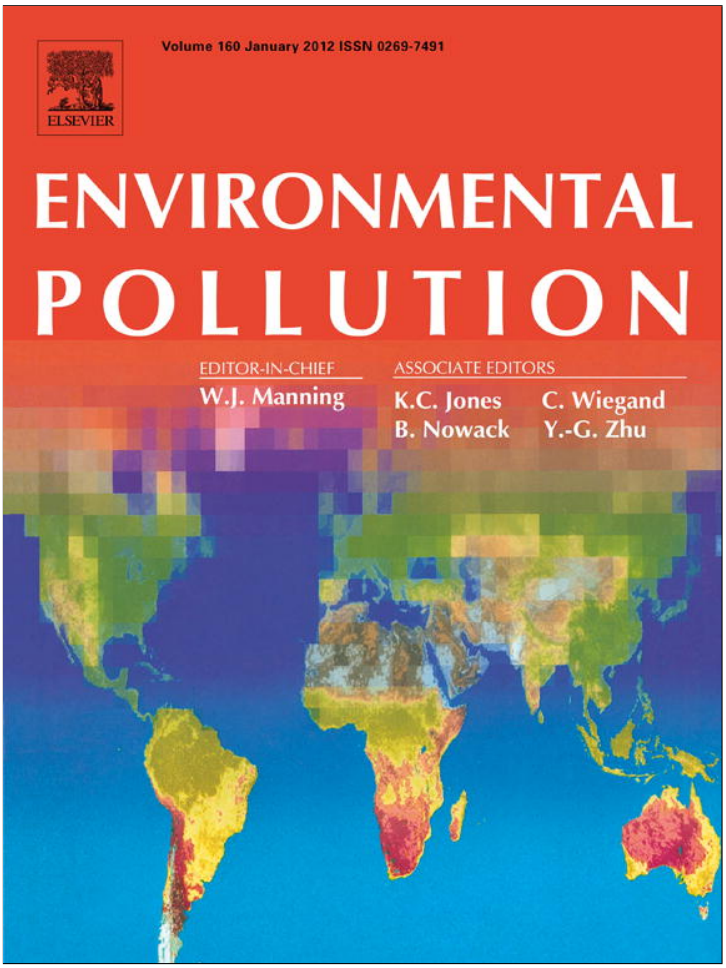

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Composition and mutagenicity of PAHs associated with urban airborne particles in Córdoba, Argentina

Hebe A. Carreras [a,*], Maria Elena Calderón-Segura [b], Sandra Gómez-Arroyo [b], Mario A. Murillo-Tovar [b], Omar Amador-Muñoz [b]

[a] Departamento de Química, Facultad de Ciencias Exactas, Físicas y Naturales, Universidad Nacional de Córdoba, Córdoba, Argentina
[b] Laboratorios de Genotoxicología y Mutagénesis Ambientales, Centro de Ciencias de la Atmósfera, Universidad Nacional Autónoma de México, Coyoacán, México D.F., Mexico

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ABSTRACT

The comet assay and micronucleous test were used to assess the genotoxicity of organic compounds associated with particulate material collected in the city of Córdoba, Argentina. Samples were collected on fiber glass filters and their organic extracts were analyzed by GC-MS. These extracts were used for the comet assay on human lymphocytes and for the MCN test with *Tradescantia pallida*. The concentrations of polycyclic aromatic hydrocarbons as well as some of their nitro derivates were higher during winter. Their composition suggested that their main emission sources were gasoline and diesel vehicles. We observed genotoxic effects of these organic extracts due to the presence of both direct and indirect acting mutagens. We found a good agreement between the two test systems employed, which encourages the further use of plant bioassays for air pollution monitoring, especially in developing countries, due to their flexibility, low cost and efficiency.

1. Introduction

Airborne particles are a worldwide problem, with most being generated as a result of industrial activities and motor vehicle exhausts. Exposure to airborne particles can result in a wide range of effects such as increased daily mortality and morbidity in adults (Schwartz et al., 1996; Pope and Kalkstein, 1996; Ostro et al., 2000), breathing difficulties and development of lung cancer (Boffetta and Nyberg, 2003; Vineis and Husgafvel-Pursiainen, 2005). In addition to the respiratory tract, vital functions of other organs may be affected as well (Cohen et al., 2005). However, the quantification of the relationship between the above-mentioned harmful effects and particles is complicated by the fact that ambient air is a complex mixture of components with variable chemical compositions and physicochemical properties, long-range transport potential, toxicity and carcinogenicity. Although toxicity may be due to a direct action of particles on the respiratory tissue, the particle composition varies extensively, and toxicological effects may also be mediated by compounds present in or associated with ambient particles (Lei et al., 2004).

In urban environments, airborne particles are usually composed of an inert carbonaceous core covered by layers of adsorbed pollutant molecules such as metals, acid salts, sulfur compounds, small organic components, and other trace materials (US EPA, 2004). This complex mixture varies depending on the season, geography, meteorological conditions and pollution sources. The organic chemicals associated with airborne particles and measured in the extractable organic matter include hundreds of compounds, among which the polycyclic aromatic hydrocarbons (PAHs) have been the most widely investigated in studies exploring the mutagenic and potentially carcinogenic activity of ambient particulate matter (Atkinson and Arey, 1994; Arey, 1998; International Agency for Research on Cancer (IARC), 1998). As only 30–40% of the organic compounds of the airborne particles have been identified, Van Houdt et al. (1987) considered that the effects of the complex mixture could give a more realistic basis of mutagenic activity than any test of its individual components because of the occurrence of synergisms or antagonisms (Alink et al., 1983).

In order to assess the genotoxicity of urban air pollution, several studies have been conducted with the majority of them using the Salmonella mutagenicity assay or Ames test. Some others have used mammalian cells by measuring DNA adducts, DNA damage, micronuclei (MN) or chromosomal aberrations. However, very few of these have measured the frequency of micronuclei in *Tradescantia*.

Córdoba is the second largest city in Argentina and several studies have shown that increases in its airborne particulate pollution can cause greater morbidity due to respiratory diseases

* Corresponding author.
E-mail addresses: hcarreras@com.uncor.edu, hebecarreras@gmail.com (H.A. Carreras).

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(Carreras et al., 2008). Indeed, previous studies have found that, in the urban area, the average PM_{10} and $PM_{2.5}$ values are almost 2.5 and 2.8 times higher respectively than the corresponding EU limit values for air quality (Lopez et al., 2011). A few research studies have revealed the mutagenic activity of air pollutants in Córdoba in a biomonitoring experimental system with *Tradescantia* (Carreras et al., 2006), but there is no other information on the components of PM that might have been responsible for this observed genotoxicity. Therefore, it is essential to clarify the nature of organic extracts obtained from PM in Córdoba.

Since many organic air pollutants are adsorbed on the surface of airborne particles, and some of the components are known or suspected human carcinogens, we collected air samples during different seasons in Córdoba city. Here, we report on the results from the comet bioassay in human lymphocytes and on the micronucleous assay in *Tradescantia* pollen mother cells, which identify the genotoxic potency of airborne particles in Córdoba.

2. Materials and methods

2.1. Sampling

Total suspended particles (TSP) were collected on glass fiber filters using a medium volume sampler with a flow rate of 0.2 m^3 min^{-1}. The collector was placed 7 m high on the roof of the Chemistry Department at the FCEFyN, Córdoba University during the following periods March–April, June–July and November–December, 2008. Each sample was obtained in 24 h sampling periods which were spaced from 7 to 12 days apart from each season. Before sampling, the filters were baked at 180 °C for at least 24 h, after which they were transferred to a chamber with constant humidity at 20–23 °C for another 24 h for conditioning. Afterwards, the filters with particles were equilibrated in the chamber for an additional 24 h. The concentration of particles (mg m^{-3}) was determined by differences in the filter weights before and after the 24 h exposures divided by the filtered air volume.

2.2. Organic matter extraction

The solvent extracted organic matter (SEOM) was obtained using an ultrasound bath at 60 °C immersed in 30 mL methylene chloride (MC) (HPLC grade, Chromanorm), for two 30-min periods, to ensure that all compounds of interest had been extracted. In order to avoid MC evaporation (likely to take place during extraction, as it has a relatively low boiling point), and the subsequent possibility of losing the compounds of interest in the process, a cooling device was fitted over the flask's mouth containing both the sample and MC. The extracts were concentrated with a rotavapor, at 30 °C, and then evaporated under a soft nitrogen flow. The concentrates were filtered with syringe filters, brought to 1 mL, and finally stored at −4 °C until SEOM determination, fractioning and analyses were carried out.

Four 24 h extracts from the same season were pooled and put in a vial in order to form the seasonal organic mixtures. The SEOM mass concentration contained in the particles was determined according to Villalobos-Pietrini et al. (2006), using 1 mL vials previously baked at 400 °C for 24 h, until achieving constant weight. A 200 μl aliquot of the seasonal organic extract was added, and later reduced to fully dried by means of a soft nitrogen flow. Finally, the vial was weighed again on a microbalance, until attaining constant weight. The mass contained in this dried fraction was diluted in 1 mL DMSO (5%) to obtain the total SEOM in mg mL^{-1}. Then, considering the air volume sampled and the filter area used, we expressed the SEOM in μg m^{-3}.

2.3. Chemical analysis

Each pooled seasonal extract was passed through a glass column packed with 10 g of silica gel which had been previously cleaned with methylene chloride for 16 h in a Soxhlet system, before being dried and deactivated with 5% distilled water. Fractionation was carried out with 25 mL of each of the following solvents: hexane, hexane:methylene chloride (6:4), methylene chloride and methanol, with each fraction containing the following five deuterated PAHs as internal standards: naphthalene-d8 (136), acenaphthene-d10 (164), phenanthrene-d10 (188), chrysene-d12 (240), and perylene-d12 (264). All fractions were analyzed using a gas chromatograph-mass spectrometer (GC-MS) (Agilent Technologies, model 6890-5973N) with a quadruple mass filter and an autosampler model 7683. In this system, electron impact mode (70 eV) was used with selected ion monitoring in order to identify and quantify PAHs in a 30-m HP5-MS capillary column (0.25 mm i.d., 0.25 mm film thickness). The oven temperature program was operated as follows: 80 °C for 2 min followed by increases of 5 °C min^{-1} until reaching 300 °C. Helium was used as carrier gas at a flow rate of 1 mL min^{-1}. Splitless injection was applied for 1 min at 300 °C. The following PAH were analyzed: FA: fluoranthene (FLAN), pyrene (PYR), benzo(a)anthracene (BaA), the sum of chrysene and triphenilene (CHR + TPH), benzo(k,j)fluoranthene (BkjF), benzo(a) pyrene (BaPY), indeno(1,2,3-cd)pyrene (IPY), dibenzo(a,h)anthracene (DBahA) and benzo(ghi)perylene (BghiP). In addition, the following nitro-PAHs were also analyzed: 1-Nitronaftaleno, 2-Nitronaftaleno, 2-Nitrobifenilo, 3-Nitrobifenil, 9-Nitroantraceno, 9-Nitrofenantreno, 3-Nitrofenantreno, 1,8-Dinitronaftaleno, 2-Nitrofluoranteno, 3-Nitrofluoranteno, 4-Nitropireno, 1-Nitropireno, 2-Nitropireno, 7-Nitrobenzo[a] antraceno, 6-Nitrocriseno, 3-Nitrobenzantrona, 6-Nitrobenzo[a]pireno, 1-Nitrobenzo [e]pireno and 3-Nitrobenzo[e]pireno. Relative response factors were determined for standard compounds in each class and used for quantitative analysis, on the basis of the same target ions (m/z). Eight-point calibration curves were obtained for all PAHs, ranging from 8 to 4500 pg mL^{-1}, ($R = 40.99$, $p < 0.001$). PAH detection limits were found to be between 3 and 39 pg m^{-3}.

2.4. Meteorological parameters

Meteorological data were obtained from the meteorological station of the National Meteorological Service located at the airport of the city of Córdoba, 7 km north from the city center (−31.31° S, −64.21° W, altitude 484 masl). Thus we investigated the influence of mean temperature, relative humidity (H), atmospheric pressure (P) and wind speed (W).

2.5. Genotoxic analysis

2.5.1. Tradescantia-micronucleus assay

The Trad-MCN assay was conducted as described by Ma et al. (1994) with slight modifications. We employed the species *Tradescantia pallida* (Rose) Hunt. var. purpurea Boom, due to its natural resistance and its easy propagation, as well as its demonstrated sensitivity to environmental mutagens (Suyama et al., 2002; Carvalho-Oliveira et al., 2005). Briefly, 12–15 cuttings bearing young inflorescence were exposed over 8 h to the seasonal organic extracts resuspended in dimethyl sulfoxide (DMSO) and tap water. As a positive control, we exposed inflorescences in solutions of trichloromethane (20 mM) and as a negative control inflorescences were exposed in DMSO and in tap water. After the exposition inflorescences were rinsed with distilled water and recovered in tap water for 24 h. Over the entire course of exposure and recovery (32 h), aeration was provided to avoid possible oxygen depletion in the solution. After recovery, 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, with only preparations containing early tetrads being considered. At least ten slides were examined for each treatment with three-hundred tetrads being 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 codes were only revealed after completing the entire experiment.

2.5.2. Comet assay

These experiments were carried out using human peripheral lymphocytes which had been isolated from whole blood samples. Twenty milliliters of heparinized venous blood, obtained from a healthy volunteer donor, was centrifuged at 2500 rpm for 20 min. The cellular layer was diluted at 1:1 with HBSS, before being placed over a Ficoll–Paque layer and centrifuged at 1500 rpm for 10 min. The lymphocytes were subsequently collected and washed twice in RPMI 1640 medium by centrifugation at 1500 rpm for 10 min, with the lymphocyte pellet being kept in RPMI 1640 medium (37 °C) supplemented with 1% penicillin/streptomycin and the cellular viability being immediately quantified in a Neubauer chamber. After this stage, cells were immediately used for the mutagenicity experiments.

The lymphocyte viability for all experimental groups before and after treatment was estimated by the trypan blue exclusion test (Altman et al., 1993). A mix of 10 μl cell pellet plus 10 μl trypan blue was incubated for 3 min and then the number of dead cells among live ones was quantified for 100 consecutive cells.

The isolated cells ($1 \cdot 10^6$ for each treatment) were exposed to a mixture of RPMI 1640 medium and the SEOM from each sampling period, for three different concentrations: 20 μl, 40 μl and 80 μl, for 24 h at 37 °C. As a negative control, lymphocytes were exposed in culture medium plus DMSO (0.5%), while as a positive control, cells were exposed in culture medium, DMSO (5%) and nitrofluorene (80 μM).

In order to test the mutagenic activity of the metabolic derivates of the SEOM we placed these extracts in presence of liver enzymes in order to mimic the normal metabolic process of metabolism (metabolic activation system). Thus, we prepared the same set of treatments with the addition of 100 μl S9 enzymatic mixture from rat liver (S9 fraction, 1 M G6P, 0.4 M $MgCl_2$, 1.65 M KCl, 0.1 M NADP, 0.2 M Na_2HPO_4/NaH_2PO_4, pH 7.5) to a final volume of 1 mL at 37 °C for 24 h. The negative control was human peripheral lymphocytes (1×10^6 cells) plus DMSO (0.5%) and 100 μl S9 enzymatic mixture in RPMI 1640 medium. The positive control was human peripheral lymphocytes plus 80 μM BaPY and 100 μl S9 in RPMI 1640 medium under the same conditions. After the treatments, the cell pellet was washed twice with RPMI 1640 medium, before performing the alkaline comet assay.

The alkaline comet assay was performed according to Speit and Hartmann (2005) and Tice et al. (2000). The lymphocytes were mixed with 90 μl low

melting point agarose (0.5%) at 37 °C, placed on fully frosted slides (Fisher) with a thin layer of normal melting point agarose (1%) and covered with a coverslip. Two slides were prepared for each SEOM concentration and the controls. The slides were kept at 4 °C for 5 min for solidification of the agarose, after which the coverslip was carefully removed, and the slides immersed in a coplin staining jar with a freshly prepared cold lysis solution (2.5 M NaCl, 100 mM EDTA, 10 mM Tris, 1% Triton X-100 and 10% DMSO, pH 10) at 4 °C for 1 h. Next, the slides were placed in a horizontal electrophoresis chamber with freshly prepared cold electrophoresis alkaline buffer (300 mM NaOH, 1 mM EDTA pH 13) for 20 min to unwind the DNA, and electrophoresis was carried out at 25 V and 300 mA for 20 min in darkness to prevent the occurrence of additional DNA damage. Subsequently, the slides were washed three times with freshly prepared neutralization buffer (0.4 M Tris, pH 7.5) for 5 min, fixed with cold absolute methanol for 5 min and air-dried at room temperature. To stain the DNA, 50 μl ethidium bromide (20 μg/mL) was added to each slide, which were then labeled with an unknown code for the viewer and examined with an Axiostar Plus Carl Zeiss fluorescent microscope equipped with an excitation filter of 515–560 nm and a barrier filter of 590 nm. To visualize the DNA damage, slides were observed at 40X magnification using a micrometric eyepiece/objective combination (1 unit = 2.41 μm at 40X magnification). The following two parameters were recorded to determine the genotoxic effect: a) frequency of comets (nuclei with DNA damage) in 50 randomly selected nuclei on each slide (two slides per treatment and controls), b) the comet tail length which was evaluated in micrometer (from the nuclear region to the end of the tail) in 100 consecutive nuclei (Fig. 1). Damaged cells were visually allocated to five categories according to comet tail size and the proportions in each category were counted. The categories were: Level 0, no tail or undamaged nucleus; Level 1, tail 1–50 l m; Level 2, tail 51–100 l m; Level 3, tail 101–150 l m, and Level 4, tail 151–200 l m.

2.6. Statistical analysis

Statistical analysis was performed using the SPSS v9.0 statistical software, and the significance level was set at 5%. The analysis of variance (ANOVA) and LSD test were used to determine significant differences between the organic extracts employed to expose the lymphocytes. In addition, regression coefficients were calculated between the comet frequencies and the concentration of organic extracts.

3. Results and discussion

The concentration of total suspended particles was higher during winter (113.0 ± 7.3 μg/m^3) than fall (90.59 ± 13.44 μg/m^3) or summer (79.0 ± 10.98 μg/m^3). The same trend was observed for extractable organic matter, which was significantly higher in samples collected during wintertime (3.17 ± 0.78 μg/m^3) than during fall (1.79 ± 0.57 μg/m^3) or summer (1.14 ± 0.45 μg/m^3; Fig. 1). Interestingly, we observed that the percentage of SEOM that represented the TSP in winter was nearly two-fold greater than that of summer (2.8% vs 1.4%), indicating that the proportion of solvent extracted organic matter in TSP varied with the season and that these particles were mostly conformed by non-solvent extracted organic matter, inorganic compounds or elemental carbon, depending on the season, as found in other studies (López-Veneroni, 2009; Mugica et al., 2009, 2010). This is consistent with the fact that strong inversions occur during winter due to long nights, dry air and cloudless skies. This allows pollutants and aerosols to be trapped at layers lower than 200 m, thus leading to high concentrations of several species and consequent adverse effects on health (Lopez et al., 2011). In fact, the low visibility values measured in winter suggest an increased content of particles in the atmosphere during this period (Table 1).

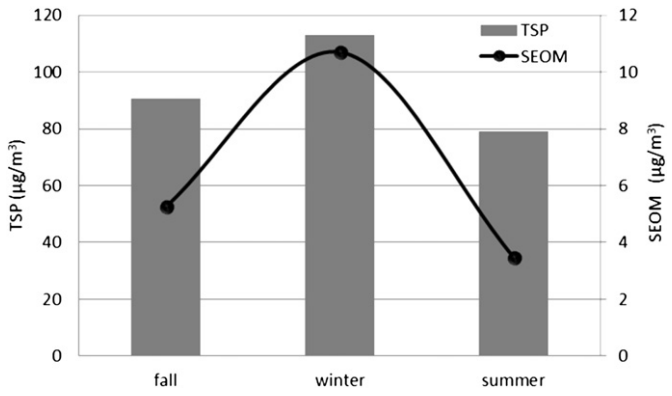

Fig. 1. Seasonal mean concentrations of total suspended particles (TSP) and extractable organic matter (EOM) collected in Córdoba city during 2008.

Table 2 shows the mean PAH concentrations in SEOM recorded in the summer, fall and winter period. We observed high concentrations of total PAHs during wintertime and fall, while during summer there was a strong decrease in these levels. The PAH composition had the fingerprint of motor vehicle emissions with a large percentage of gasoline-fuelled cars. We discarded emissions related to heating because in urban areas natural gas is employed for this purpose. Moreover, since most motor vehicles in Argentina are non-catalyst-equipped with old-fashioned engines, an increase of the budget of carbon particle emission would be expected. The high concentrations of PAHs in winter and fall may be attributed to less dispersion, due to meteorological conditions rather than to increased traffic volumes. Indeed, during fall and winter, low wind speeds, thermal inversion conditions and low temperatures make the atmosphere more stable. Thus, under these atmospheric conditions, the pollutant dispersion is least leading to accumulation and more gas-to-particle conversion which in turn leads to a higher concentration of PAHs (Sharma et al., 2007). Conversely, during summertime, precipitations, high wind speed and high temperatures along with enhanced photodecomposition of PAHs, result in increased dispersion and a decreased concentration of PAHs. Similar deposition trends with a 60% decrease in the PAH concentration due to precipitation have been reported by Kaupp and McLachlan (1999) and Ohura et al. (2004).

The presence and concentration of some PAHs were used to derive information about the relative impact of aerosols sources. In fact, both the prevalence of oil versus gasoline-fuelled cars and the relative contribution of motor vehicles, domestic heating and other urban sources have been related to different compositions of PAHs fractions (Freeman and Catell, 1990; Nielsen, 1988). Since methane and natural gas are largely used for domestic purposes, traffic is likely to be the main source of PAHs in Córdoba city. Indeed, Stein and Toselli (1996) and Olcese and Toselli (2002) have already confirmed that the main sources of air pollution in the downtown area of Córdoba city are vehicles, which release mainly primary pollutants. In agreement with this, we found that benzofluoranthene, which in urban environments is strongly related to diesel and gasoline motor vehicles, was the most abundant PAH in all samples, followed by benzo(a)anthracene and benzo(a)pyrene. Therefore, if we consider that high molecular weight PAHs have been described as tracers of gasoline-powered vehicles, while light PAHs have been found predominantly in diesel vehicle emissions (Amador-Muñoz et al., 2010), we can suggest that the main emission sources of PAHs in Córdoba city are gasoline-powered vehicles.

The levels of benzo(a)pyrene are often used as an indicator of PAHs, since they are regarded by WHO (1987) as being a good index for the whole PAH carcinogenicity. Referring the concentrations to volume of air passed, we found these to range from 0.59 ng/m^3 in autumn to 1.24 ng/m^3 in winter, which are near the maximum permissible risk level of 1 ng/m^3 benzo(a)pyrene in ambient air proposed by the European directive (2004/107/CE). In addition, we calculated the benzo(a)pyrene-equivalent carcinogenic power (BaPE) (Cecinato et al., 1998), which is an index that has been introduced for better parameterizing aerosol carcinogenicity related to the whole PAH fraction instead of just benzo(a)pyrene. In fact, this compound is easily decomposed in reactive air parcels by both light and oxidants, and when photochemical smog occurs its

Table 1
Monthly means of meteorological parameters measured during the study period in Córdoba city, 2008.

	Mean T °C	Max T °C	Min T °C	Pressure hPa	Humidity %	Precipitation mm	Visibility km	Wind km/h
Fall	19.4	26.0	13.2	1010.0	68.1	126.2	10	11.5
Winter	10	22.8	7.6	1018.7	49.4	6.0	8	5.7
Summer	22.4	30.0	17.0	1011.8	57.6	269.9	10	11

concentration does not reveal the PAH pattern. BaPE was calculated using the following formula reported by Cecinato (1997):

$$\text{BaPE} = \text{BaA}*0.06 + \text{BFa}*0.07 + \text{BaPY} + \text{DBA}*0.6 + \text{IPY}*0.08$$

We found that this ratio was two times higher in samples collected during wintertime than during fall, which indicates that in that period there was a higher risk of exposure to carcinogenic compounds. In addition, our results indicated that benzo(a) anthracene and benzo(a)pyrene were the compounds that contributed most to the value of this index, hence playing an important role in the total carcinogenicity of PAHs in the city of Córdoba.

The U.S. Environmental Protection Agency has identified 16 unsubstituted PAH as priority pollutants, from which the following seven are considered to be probable human carcinogens (benzo(a) pyrene, benzo(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, dibenzo(a,h)anthracene, and indeno(1,2,3-cd)pyrene). In the present study, the maximum concentrations of four of these were within the range of samples from European rural sites, while the maximum concentrations of benzo(a)anthracene, benzo(b)fluoranthene and indeno(1,2,3-cd)pyrene were within the range of measurements from European urban sites (Table 3). Moreover, data for the PAH indicator parameter, benzo(a)pyrene, were within the range measured in Florence, Italy (0.25–3.50 ng/m^3); Bangkok, Thailand (0.18–2.44 ng/m^3); Dusseldorf, Germany (0.66–3.15 ng/m^3) (Fernandes et al., 1999) and Mexico DF (0.08–2.1 ng/m^3, Villalobos Petrini et al., 2006).

In addition, we calculated the concentration of PAHs on 20 m^3 to estimate the daily intake rate of PAHs by inhalation of polluted ambient air. The sum of all PAHs was 57.6 ng/day in fall, 110.2 ng/day in winter and 2.8 ng/day in summer. All these values were much lower than the median value of 160 ng/day estimated by Menzie et al. (1992) as a daily potential dose of carcinogenic PAH.

The direct acting mutagenic effects have been previously related to nitro-hydroxy- and methoxy-PAH derivatives, produced by partial transformations of PAHs into products of oxidation and nitration (Pitts et al., 1978), as well as of nitro-PAH delivered by emission sources (Nielsen, 1984). Arey et al. (1992) found that nitro-PAH are strong direct acting mutagens which contribute up to 10% of the overall direct mutagenicity of SEOM. Therefore, we also analyzed the nitro-PAH composition of the organic extracts corresponding to each sampling period (Table 4). We found that most of the nitro-PAHs had almost the same concentration all over the seasons, except for the concentrations of 9-Nitroanthracene, 9-Nitrophenantrene, 3-Nitrophenantrene, 2-Nitrofluoranthene, 4-Nitropyrene, 1-Nitropyrene and 2-Nitropyrene which were higher in the winter extracts. The increase of the nitro-PAH concentration levels in the cold–dry season may be related, as it was mentioned before, to climatic conditions due to the frequent thermal inversions occurring throughout the season (Mastral et al., 2003). Thus, the accumulation of pollutants is favored when inversions take place coupled with low wind speed, due to the absence of vertical dispersion brought about by the relative stability of the warm air layer (Guzmán Torres et al., 2009). In agreement with our results, Valle Hernandez et al. (2010) also found that 9-Nitroanthracene followed by 3-Nitrophenanthrene and 9-Nitrophenanthrene were among the most abundant nitro-PAHs associated with particles in northern Mexico city. Regarding the emission sources of the measured nitro-PAHs, two of the above-mentioned compounds more abundant during winter, 1-Nitropyrene and 3-Nitrofluoranthene have also been observed in diesel engine exhaust gases, which strongly suggest these to be their main emission source (Albinet et al., 2007). Indeed, 1-Nitropyrene has been considered to be the marker nitro-PAH of the diesel engine exhaust gases, because it is only emitted directly (Arey, 1998; Albinet et al., 2007). Therefore, its presence in the atmosphere is a sign of pollution due to vehicle emissions.

Although studies on nitro-PAHs are somewhat scarce, we compared our results with data from other Latin-American urban areas: Sao Paulo (De Castro et al., 2008) and Mexico DF (Valle Hernandez et al., 2010). We found similar or lower concentrations occurred for most of the compounds measured, except for 3-

Table 2
PAH* seasonal mean concentrations (ng/m^3) in Córdoba city, 2008.

	Fall		Winter		Summer	
	Mean	SD	Mean	SD	Mean	SD
FLAN	0.05	0.01	0.15	0.02	0.00	0.00
PYR	0.07	0.00	0.12	0.49	0.00	0.00
BaA	0.63	0.01	1.47	0.30	0.00	0.00
CHR + TPH	0.30	0.03	0.56	0.29	0.00	0.00
BkjF	0.70	0.08	1.60	1.54	0.00	0.00
BaPY	0.59	0.12	1.24	0.73	0.00	0.00
IPY	0.19	0.02	0.37	0.48	0.00	0.00
DBahA	0.00	0.00	0.00	0.00	0.00	0.00
BghiP	0.36	0.07	0.00	0.00	0.14	0.01
Total	2.88		5.51		0.14	
BaPE	0.69		1.47		0.00	
BaPE/BaPY	1.17		1.18		0.00	

*PAH identification: FLAN: fluoranthene; PYR: pyrene; BaA: benzo(a)anthracene; CHR + TPH: sum of chrysene and triphenilene; BkjF: benzo(k,j)fluoranthene; BaPY: benzo(a)pyrene; IPY: indeno(1,2,3-cd)pyrene; DahA: dibenzo(a,h)anthracene; BghiP: benzo(ghi)perylene; Total: sum of PAHs; BaPE: benzo(a)pyrene-equivalent of carcinogenic potency.

Table 3
Comparison of recent typical European PAH concentrations[a] in ng/m^3 as annual mean values with maximum values obtained in the present study.

PAHs*	Remote site	Rural site	Urban	Traffic	Industrial	Present study
FLAN	0.14	0.04–7.4			42	0.153
PYR	0.08	0.1–6.1	0.24–1.2	9.2–15	75	0.116
BaA	0.00 –0.02	0.01–0.9	0.2–1.3	0.6–4.2	0.37–42	1.471
CHR + TPH						0.557
BkjF	0.04 –0.32	0.2–1			0.3–17	1.602
BaPY	0.02	0.02–1.6	0.4–2	0.7–3.1	0.5–39	1.242
IPY	0.02 –0.04	0.04–0.21	0.3–2.1	1.3–2.6	0.4–37	0.365
BghiP	0.01	0.15–1.0	0.5–2.8	1–4.7	0.7–52	0.356

*PAH identification: FLAN: fluoranthene; PYR: pyrene; BaA: benzo(a)anthracene; CHR + BlPH: sum of chrysene and triphenilene; BkjF: benzofluoranthene; BaPY: benzo(a)pyrene; IPY: indeno(1,2,3-cd)pyrene; BghiP: benzo(ghi)perylene.
[a] Ambient Air Pollution by PAH. Position Paper Annexes, 2001.

Table 4
Nitro-PAH seasonal mean concentrations (pg m^{-3}) in Córdoba city, 2008.

	Fall		Winter		Summer		Mexico city[a]	Sao Paulo[b]
	Mean	SD	Mean	SD	Mean	SD		
1-Nitronaphtalene	18.09	0.00	17.29	0.00	18.96	0.00		
2-Nitronaphtalene	16.62	0.00	15.89	0.00	17.43	0.00		
2-Nitrobiphenyl	14.18	0.03	13.55	0.00	14.87	0.00		
3-Nitrobiphenyl	21.05	0.39	23.87	5.71	22.11	0.51		
9-Nitroanthracene	28.07	0.91	56.50	3.98	38.53	1.06	41.45	114
9-Nitrophenantrene	21.04	0.00	42.80	0.26	25.46	1.07	2.69	28
3-Nitrophenantrene	30.69	0.00	45.09	0.85	35.13	1.19	21.50	29.3
1,8-Dinitronaphtalene	26.09	0.00	24.93	0.00	27.35	0.00		
2-Nitrofluoranthene	21.09	0.00	38.40	2.95	26.70	0.64	52.10	49.8
3-Nitrofluoranthene	26.53	0.00	25.36	0.00	27.81	0.00	n.a.	3.1
4-Nitropyrene	16.80	0.00	33.70	5.40	20.68	1.35		
1-Nitropyrene	24.57	0.00	35.74	5.26	28.93	0.79	16.17	9.7
2-Nitropyrene	29.54	0.00	47.31	26.98	30.96	0.00		
7-Nitrobenzo[a] anthracene	23.04	0.00	27.58	7.86	28.40	0.56	n.d.	15.3
6-Nitrochrysene	15.40	0.00	14.72	0.00	16.14	0.00	n.a.	1.8
3-Nitrobenzanthrone	96.49	0.00	92.22	0.00	101.15	0.00		
6-Nitrobenzo(a)pyrene	36.89	31.50	35.26	0.00	38.67	0.00		
1-Nitrobenzo(e)pyrene	22.27	0.00	42.58	0.00	46.70	0.00		
3-Nitrobenzo(e)pyrene	18.09	0.00	17.29	0.00	18.96	0.00		

[a] Valle Hernández et al., 2010.
[b] De Castro et al., 2008.

Nitrofluoranthene, 1-Nitropyrene, 7-Nitrobenzo[a]antraceno and 6-Nitrocriseno whose concentrations were at least twice as high as the ones measured in Sao Paulo and Mexico city. It is of concern that these compounds are considerably high since 1-Nitropyrene and 6-Nitrocriseno have been individually classified by the IARC as being possibly carcinogenic to humans and inducers of other relevant biological effects as well (IARC, 1989). Moreover, exposure to low concentrations of 1-Nitropyrene induces DNA damage and increased levels of reactive oxygen species. In addition, some of its metabolites have been identified in the urine of non-smoking healthy inhabitants of urban areas, which is strong evidence that regular exposure to urban pollutants, where nitro-PAHs associated with airborne particulate matter are usually present, is the main incorporation pathway for humans (Toriba et al., 2007).

Genotoxicity data from the comet assay are presented in Fig. 2. Human lymphocytes were exposed to increasing concentrations of SEOM collected in Córdoba city during fall, winter and summer, with or without metabolic activation (S9). We found a slightly increased frequency of comets when samples were treated with organic extracts plus metabolic activation. However the high percentage of damaged cells in both treatments suggests the presence of direct as well as indirect acting mutagens. Barale et al. (1991) and Villalobos Petrini et al. (1995) also found that the mutagenic activity was higher in samples treated with suspended particles and S9. For samples treated with fall extracts the frequency of comets rose steadily as the amount of SEOM increased, while there was almost no difference between the SEOM concentrations in samples treated with extracts collected during winter or summer. Moreover, the regression analysis, with comet frequencies as a function of the amount of SEOM applied, showed high regression coefficients only in samples treated with fall extracts, with or without S9 ($R^2 = 0.92$ with S9 and $R^2 = 0.80$ without S9), while the coefficients for the other treatments were much lower ($R^2 = 0.60$, data not shown).

The analysis of comet tail length from lymphocytes treated with organic extracts (Table 5a) showed significantly longer tails in cells exposed to extracts from all seasons compared to the negative control, except for the less concentrated fall extract. These samples showed a steady increase in tail length as the concentration of SEOM increased, and most of the cells were categorized as low or medium DNA damage. On the other hand, cells that were treated with medium or high concentrations of winter extracts fell mostly in the categories with the largest DNA damage (twice that of the positive control) while cells exposed to the lowest winter concentration revealed damage similar to this control. Cells treated with

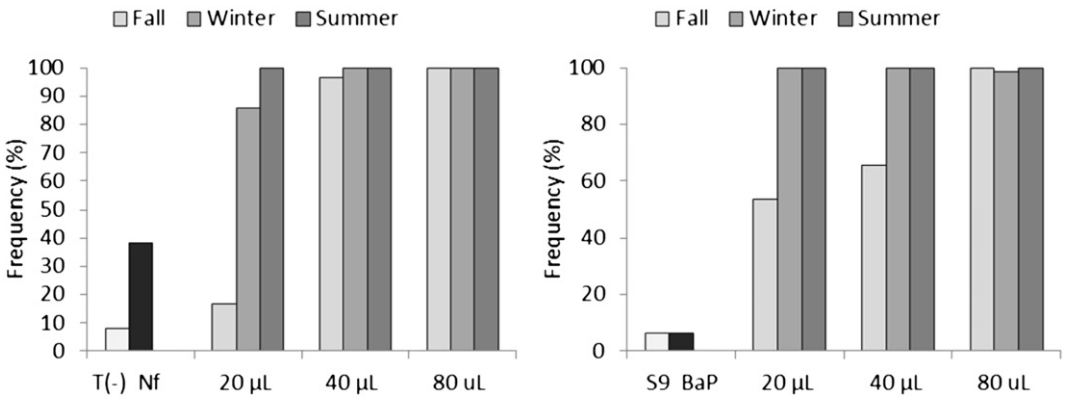

Fig. 2. Frequency of comets (%) in lymphocytes exposed to organic extracts collected during fall, winter and summer in Córdoba city, with (right panel) or without metabolic activation (left panel).

Table 5
DNA damage evaluated by alkaline comet assay in human peripheral lymphocytes induced by extractable organic matter without (a) or with (b) metabolic activation.

	Length			Level of DNA damage				
	N	Mean	SD	L0	L1	L2	L3	L4
(a)								
Negative control	207	2.43 aA*a*	0.59	88.4	10.63	0.97	0	0
Positive control	154	50.4 cB*d*	1.57	0	50	50	0	0
Fall***								
20 μl	48	2.31 a	0.89	83.33	16.67	0	0	0
40 μl	91	25.1 b	1.59	13.19	84.62	2.2	0	0
80 μl	59	84.0 d	4.07	0	15.25	61.02	23.73	0
Winter***								
20 μl	57	56.8 B	2.21	3.57	16.07	80.36	0	0
40 μl	50	116 D	4.51	0	0	32	58	10
80 μl	71	97.7 C	2.88	0	4.23	46.48	49.3	0
Summer***								
20 μl	53	23.8 *b*	1.16	0	100	0	0	0
40 μl	68	29.1 *c*	2.57	0	85.29	14.71	0	0
80 μl	100	72.6 *e*	1.14	0	9	91	0	0
(b)								
Negative control	155	2.38 aA*a*	0.81	90.32	8.39	1.29	0	0
Positive control	239	28.26 bB*b*	1.35	18.83	72.8	8.37	0	0
Fall***								
20 μl	99	28.75 b	3.15	46.46	20.2	33.33	0	0
40 μl	99	44.33 c	4.05	34.34	16.16	44.44	5.05	0
80 μl	51	42.55 c	1.18	0	86.27	13.73	0	0
Winter***								
20 μl	33	136.86 C	9.65	0	0	18.18	30.33	45.45
40 μl	58	133.68 C	4.23	0	0	0	48.26	32.76
80 μl	73	162.26 D	6.04	1.37	18.18	0	23.29	61.64
Summer***								
20 μl	92	33.04 *b*	0.97	0	97.83	2.17	0	0
40 μl	61	49.03 *c*	2.41	0	55.74	44.26	0	0
80 μl	76	60.06 *d*	1.74	0	20.55	79.45	0	0

*** Anova $p < 0.001$; mean values followed by the same letter (type and font) do not differ significantly ($p < 0.05$).

summer extracts had less damage; with only those exposed to the most concentrated solution showing a tail significantly longer than the positive control and most cells being in the categories with low DNA damage.

We observed a significant increase in tail length from cells treated with the organic extracts plus the enzymatic extract S9 with respect to the negative control (Table 5b). All cells exposed to winter extracts showed significantly longer tails than the positive control. In fact, even nuclei exposed to the lowest SEOM concentration had tails four times longer than the positive control. Moreover, most of these samples were in the categories representing the highest DNA damage. Smaller increases were observed in cells exposed to fall or summer extracts, with these less concentrated treatments being similar to the positive control and the samples being in the categories with low or medium DNA damage.

Genotoxic data obtained using the MCN test are shown in Fig. 3. Similar to the trend already observed with the comet assay, we found a significantly higher frequency of MCN in the inflorescences exposed to the winter extracts, compared to samples exposed to fall or summer extracts. This result may be due to the fact that winter extracts have more genotoxic compounds and also because of the capacity of *Tradescantia* to activate promutagens, as already observed Ma et al. (1987). However, we believe the first hypothesis is much more feasible since the genotoxicity of summer and fall extracts was even lower than the genotoxicity observed in the control with DMSO. The fact that organic extracts are associated with significant changes in the spontaneous rate of micronuclei reinforces the concept that air pollutants from Córdoba city may cause malignancies in humans. Thus, even though mutagenesis in plants cannot be extrapolated to cancer risk, these plant bioassays are very useful as screening instruments for assessing human risk.

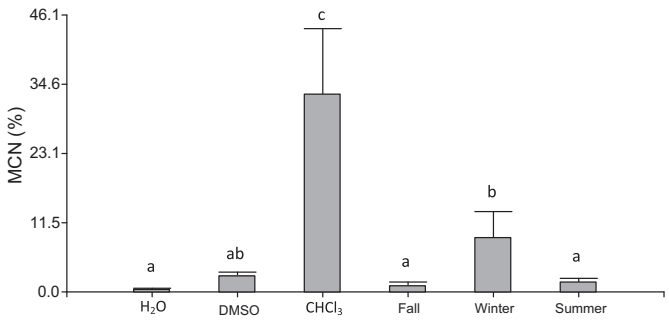

Fig. 3. MCN frequencies measured in *T. pallida* plants exposed to different seasonal organic extracts collected in Córdoba city, Argentina.

4. Conclusions

The concentrations of suspended particles and solvent extracted organic compounds were consistent with the meteorological conditions of the sampling period, with the highest values being found during the cold–dry season and the lowest values corresponding to the summer. In addition, the concentrations of PAHs and nitro-PAHs were associated with the characteristics of vehicle emissions, which were their main emission sources. The most abundant PAHs were benzofluoranthene, benzo(a)anthracene and benzo(a) pyrene, while the most abundant nitro-PAHs were 3-Nitrobenzanthrone, 9-Nitroanthracene, and 2-Nitropyrene followed by 3-Nitrophenantrene and 9-Nitrophenantrene. The fact that some of these compounds have been previously reported as carcinogenic and mutagenic in experimental in vivo models, suggests that they might also be involved in the observed genotoxic effects in lymphocytes and *T. pallida* cells. Therefore, it is highly relevant to

intensify studies on the atmospheric concentrations of PAHs and nitro-PAHs since they can aid in the surveillance of exposure and the subsequent associated cancer risk.

The genotoxic effects observed after exposure to the organic extracts of airborne particles were due to the presence of both direct and indirect acting chemical mutagens. However, the measurement of comet tail length that allows a more precise DNA damage analysis, indicated that indirect mutagens or promutagens were possibly more abundant than direct mutagens, particularly in winter extracts.

We found a rather good agreement between the outcomes of the comet assay and the MCN test, revealing that organic compounds may be responsible for either permanent or reparable DNA damage. Any difference could be due to intrinsic characteristics of the two test systems: in the comet assay, the compounds administered are largely available in the culture media and reach the target cells, whereas in the MCN test, the compounds are absorbed by the xylem and then transferred to the target cell. Hence, their toxicity might be reduced due to oxidation, transformation or even compartmentalization of the xenobiotic.

The results obtained with the comet assay are much more easily extrapolated to genetic damage in humans. However, studies like the present one, which revealed a relationship between genetic damage in *Tradescantia* and ambient concentrations of known human carcinogens (PAHs and nitro-PAHs), encourages the use of plant bioassays for air pollution monitoring not only because of their flexibility, low cost and efficiency, but also as they reveal the effects of pollutants on a living organism.

Considering that the levels of several measured organic compounds were higher than the ones found in other urban areas and that these compounds have also been reported as carcinogenic and mutagenic, strong action should be taken in order to reduce the human exposition to vehicle emissions by introducing abatement devices on vehicles or limiting circulation. Moreover if we consider that genotoxic effects might be even worse than the ones observed in the present study, since the measured concentrations of organic compounds were possibly underestimated. At present, there is no regulatory norm which establishes limits of PAH concentrations either in Argentina or in Córdoba city; the only regulation being that the concentration of PM_{10} should not exceed 150 $\mu g/m^3$. However, our results suggest that even below these concentrations, airborne particles could be a serious risk for human health. Therefore, the information provided in the present study should be considered for the improvement of such regulations.

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