Seasonal variations in the level of mutagenicity: an assessment of respirable particulate matter in Rio de Janeiro, Brazil

C. R. Rainho[1], S. M. Corrêa[2], J. L. Mazzei[3], C. A. F. Aiub[4] & I. Felzenszwalb[1]
[1]*Department of Biophysics and Biometry, State University of Rio de Janeiro, Brazil*
[2]*Department of Environmental Chemistry, State University of Rio de Janeiro, Brazil*
[3]*Farmanguinhos Platform for Analytical Methods, Fundação Oswaldo Cruz, Brazil*
[4]*Department of Genetics and Molecular Biology, Federal University of the State of Rio de Janeiro, Brazil*

Abstract

Respirable particles (PM2.5) can become associated with organic matter containing several compounds such as polycyclic aromatic hydrocarbons (PAHs). Many PAHs have been identified as cancer-inducing chemicals. The mutagenicity of airborne particles is generally associated with PAHs, but recent reviews show that PAHs may not be the predominant mutagens in atmospheric pollution, and that nitroaromatic compounds, aromatic amines and aromatic ketones, often found in moderately polar or highly polar organic fractions, are potent mutagens. Nitro-polycyclicaromatics (nitro-PAHs) are persistent environmental mutagens and can be found in airborne suspended particles from direct sources such as diesel and gasoline exhausts, or may be products of atmospheric reactions in the presence of NO_2 and NO_3 radicals. In the present work we compared PAH levels and mutagenicity using gas chromatography spectrometry and the Salmonella/microsome assay on organic extracts of PM2.5 for *Salmonella typhimurium* strain TA98. The samples were collected in two periods: (I) July to October 2010 and (II) November 2010 to May 2011 at three sites in Rio de Janeiro – (1) low urban traffic at the University campus; (2) heavy

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urban traffic at Brasil Avenue and (3) Rebouças tunnel. We also performed measurements of nitro-PAH levels for November 2010 to May 2011. For both periods, site 3 showed the highest concentrations of PAHs and nitro-PAHs. Period I showed the higher values of rev/m^3. Mutagenic frameshift responses in the absence of metabolic activation were detected at all the sites in periods I and II. In the presence of metabolic activation this response was observed for all three sites in period I, but only for site 3 in period II. Nitroarenes and dinitroarenes were detected at all three sites in period I. In period II, the presence of nitroarenes was also detected at all sites, but dinitroarenes were only detected at sites 2 and 3. The information generated in this study shows that different levels of PAHs and nitroderivatives, influenced by seasonal variations in climatic conditions, probably contribute to the detected airborne mutagenicity.
Keywords: seasonal variations, mutagenicity assessment, respirable particulate matter, polycyclic aromatic hydrocarbons, nitro-polycyclicaromatics.

1 Introduction

Respirable particles ≤2.5 μm (PM2.5) originating from motor vehicle exhaust fumes are a major source of particulate matter, with potentially serious health impacts [1]. These pollutants can become associated with organic matter containing several compounds, such as polycyclic aromatic hydrocarbons (PAHs) [2]. Many PAHs have been identified as cancer-inducing chemicals [2]. Recent reviews show that PAHs are not the predominant mutagens in atmospheric pollution. Nitroaromatic compounds, aromatic amines and aromatic ketones, often found in moderately polar or highly polar fractions, are potent mutagens [3, 4]. Nitro-polycyclicaromatics (nitro-PAHs) are persistent environmental mutagens and can be found in suspended airborne particles from direct sources such as diesel and gasoline exhaust fumes, or may be products of atmospheric reactions in the presence of NO_2 and NO_3 radicals [4–6].

In Rio de Janeiro, climatic conditions vary during the year, with precipitation occurring mainly between the months of December and March (rainy season) and scarcely at all in July and August (dry season) [7]. Critical air pollutants, such as PAHs and PM, increase during the dry season and decrease during the rainy season [8]. In 2010, we conducted a monitoring study at three sites of the city of Rio de Janeiro: the campus of the Rio de Janeiro State University, Avenida Brasil and Rebouças tunnel [9, 10]. In these studies we detected values for PM2.5 that exceeded the values established by the World Health Organization [11] at all three sites. Furthermore, mutagenicity was detected in the organic extract of PM2.5 for *Salmonella typhimurium* strain TA98 and derivatives with sensitivity to nitro compounds [9, 10].

To study the influence of seasonal variations on the mutagenic activity of PM samples, we analyzed samples collected between November 2010 and May 2011 at the same sites and compared them with the data from our previous studies [9, 10].

2 Materials and methods

2.1 Sampling sites

The samples were collected at three sites in Rio de Janeiro: the campus of the Rio de Janeiro State University (site 1), Avenida Brasil (site 2) and Rebouças tunnel (site 3) between November 2010 and May 2011 (Period II). Site 1, with low traffic, is located in a residential area of the city's north zone. Site 2 has heavy traffic (~250,000 vehicles/day) and is the city's biggest highway, covering 58 km in length and crossing 27 neighborhoods. Site 3 has heavy traffic (~190,000 vehicles/day). It connects the north and south zones of the city and is 2.8 km long [9, 10].

Airborne PM2.5 samples were collected on fiberglass filters (E558 X 10IN, 254mm x 203mm) using a high-volume collector (AVG MP 2.5, 1.13 m^3/mim) for 24h at sites 1 and 2, and 6h at site 3. Four monthly samplings were performed for each site. The filters were weighed and stabilized before and after samples (45% humidity) for the determination of particulate concentration, expressed in $\mu g/m^3$ units of sampled air [12–14]. At the end of the sampling, the filters were combined to form a pool sample.

2.2 Extraction of organic compounds

Half of each filter was sonicated in three rounds of 10 min each using dichloromethane (DCM, CASRN. 75-09-2, Tedia Brazil, Brazil, purity 99.9%). The extracts were concentrated to 15 mL in a rotating evaporator and filtered in a Teflon membrane (0.5 μm). The concentration of extractable organic matter (EOM, in $\mu g/m^3$) was calculated. Prior to bioassays, the organic extract was dried at 4°C and resuspended in 5 μl dimethyl sulfoxide (DMSO, CASRN. 67-68-5, Synth, Brazil, purity 99.9%) [12–14].

2.3 Analysis of polycyclic aromatic hydrocarbons (PAHs)

PAHs were identified and quantified by gas chromatography/mass spectrometry (GC/MS) using a Varian system consisting of a gas chromatograph (450-GC) with a split/splitless injector 1177S/SL (kept at 300°C) coupled to the mass spectrometer detector (MS 220). The ion trap (250ºC), manifold (280ºC) and transfer line (280ºC) were maintained at constant temperatures. PAHs were identified by mass similarity and by the retention time of the components in a commercial standard kit (Supelco, PAH610-S). Quantification was based on five calibration points, which were constructed from each standard for all the target analytes, ranging from 10 to 250 pg/μL. Injections (2.0 μL) were splitless, with the split opened after 0.5 min, and helium 5.0 was used as the carrier gas. A VF-5MS column (30 m × 0.25 mm × 0.25 μm) was employed. The column and septum purge flows were set at 1.6 and 3 mL/min, respectively. The oven temperature program was as follows: 70°C for 4 min then heating to 300°C at 10°C/min. This procedure was designed for the analysis of the 16 priority PAHs, but only six were detected: phenanthrene, fluoranthene, pyrene, benzo[a]anthracene, chrysene and benzo[a]pyrene. The limits of quantification

were determined from the minimum point in the calibration curves. Limits of detection were determined from PAH concentrations, which resulted in a signal-to-noise ratio of 3:1. The results were expressed in ng/m^3 [15].

2.4 Analysis of nitro-polycyclic aromatic hydrocarbons (nitro-PAHs)

Nitro-PAHs were identified and quantified by GC/MS using a Varian system consisting of a gas chromatograph (450-GC) with a Programmed Temperature Vaporization Injector 1079 (PTV) starting at 75°C for 0.2 min then heated at 200°C/min until 340°C. The mass spectrometer detector (MS 220) was operated under the following conditions: ion trap (250°C), manifold (280°C) and transfer line (280°C) were maintained at constant temperatures. Nitro-PAHs were identified by mass similarity and by the retention time of the components in a commercial standard kit (Supelco, PAH610-S). Quantification was based on five calibration points in duplicate, which were constructed from each standard for all the target analytes, ranging from 25 to 400 ppb. Injections (50 μL) were splitless, with the split opened after 0.5 min, and helium 5.0 was used as the carrier gas at a constant flow of 1.2 mL/min. A VF-5MS column (30 m × 0.25 mm × 0.25 μm) was employed. The oven temperature program was as follows: 70°C for 2 min then heating to 210°C at 10°C/min, then heating to 300°C at 20°C/min. This procedure was designed for the analysis of 13 nitro-PAHs: nitro-naphthalene, nitro-acenaphthylene, nitro-acenaphthene, nitro-fluorene, nitro-phenanthrene, nitro-anthracene, nitro-fluoranthene, nitro-pyrene, nitro-benzo[a]anthracene, nitro-chrysene, nitro-benzo[b]fluoranthene, nitro-benzo[k]fluoranthene and nitro-benzo[a]pyrene. The limits of quantification were determined from the minimum point in the calibration curves. Limits of detection were determined from nitro-PAH concentrations, which resulted in a signal-to-noise ratio of 3:1. The results were expressed in ng/m^3.

2.5 Salmonella/microsome assay

The organic extracts were assayed for mutagenicity using the microsuspension version [16] of the *Salmonella/microsome* assay [17]. *Salmonella typhimurium* TA98 (frameshift strain) and the derivative strains YG1021 (nitroreductase-overproducing) and YG1024 (*O*-acetyltransferase-overproducing) [18] were used, with and without metabolic activation (S9 mix fraction). Five concentrations of each sample (10, 20, 30, 40 and 50 μg/plate) were tested in triplicate. The samples were pre-incubated for 90 min. All assays were carried out under yellow light and in the presence of negative (dimethyl sulfoxide - DMSO solvent, 5 μL/plate) and positive (4-nitroquinoline oxide, 0.5 μg/plate, CASRN. 56-57-5, and 2-aminofluorene, 1 μg/plate, CASRN. 153-78-6, from Sigma-Aldrich, St. Louis, MO, USA) controls. Plates were incubated in the dark at 37°C for 72h, after which time revertants were counted. The sample was considered positive when a mutagenesis value of at least twice the negative value, a significant ANOVA ($p < 0.05$) and a positive dose–response rate ($p < 0.05$) were observed. The results of the different assays were analyzed via the SALANAL program (Salmonella Assay Analysis, version 1.0, Integrated Laboratory Systems of Research Triangle Institute, RTP, North Carolina, USA).

The choice between linear regression and the Bernstein model [19] was made to allow the elimination of data for doses outside the linear portion of the dose–response curve. Positive results were interpreted as presenting significant mutagenicity, and were expressed as the number of revertants per volume of air sampled (rev/m^3), i.e. rev/µg multiplied by EOM in µg/m^3. In the cytotoxicity test, the solution containing the sample and the bacterial culture (100-200 cells) were plated on nutrient agar plates and incubated at 37°C for 24 h and the surviving colonies were counted. The sample was considered cytotoxic if the percentage of surviving cells was less than 60% of the negative control after one or more doses [14].

3 Results

3.1 Airborne particulate matter

Table 1 shows the air volume (m^3), PM2.5 concentration ($\mu g/m^3$), and extractable organic matter (EOM) ($\mu g/m^3$) of the samples analyzed from the different sites between November 2010 and May 2011.

The highest average PM2.5 values were detected at site 3 (54 to 141 $\mu g/m^3$), followed by site 2 (27 to 38 $\mu g/m^3$) and site 1 (25 and 34 $\mu g/m^3$) (Table 1).

Table 1: Collection sites, air volume, PM 2.5 µm concentration and extractable organic matter (EOM) of the samples analyzed.

Site	Month	Air Volume (m^3) ± S.D.	PM 2.5 µm (µg/m^3) ± S.D.	EOM (µg/m^3)
1	Nov	1567 ± 112	25 ± 15	8.62
	Dec	1538 ± 25	15 ± 7	1.46
	Jan	1568 ± 50	17 ± 2	13.89
	Feb	1538 ± 12	14 ± 8	0.35
	Apr	1530 ± 11	19 ± 4	0.68
	May	1524 ± 66	34 ± 22	4.19
2	Nov	1552 ± 9	31 ± 8	8.70
	Dec	1536 ± 39	25 ± 9	3.90
	Jan	1568 ± 50	28 ± 12	6.22
	Feb	1580 ± 38	27 ± 10	10.92
	Apr	1519 ± 31	38 ± 20	3.21
	May	1490 ± 49	27 ± 11	10.73
3	Nov	379 ± 5	74 ± 24	29.66
	Dec	442 ± 10	70 ± 50	49.20
	Jan	397 ± 16	68 ± 20	54.85
	Feb	473 ± 67	62 ± 25	1.58
	Apr	415 ± 458	141 ± 44	3.49
	May	458 ± 47	54 ± 27	3.16

1 – UERJ; 2 – Avenida Brasil; 3 – Rebouças tunnel. S.D.= standard deviation. Airborne PM 2.5µm samples were collected for 24h at sites 1 and 2, and 6h at site 3. 6h time filter saturation at site 3. No collections in March.

3.2 Analysis of polycyclic aromatic hydrocarbons (PAHs)

Figure 1 shows PAH concentrations, in ng/m^3, at the three sites during period II.

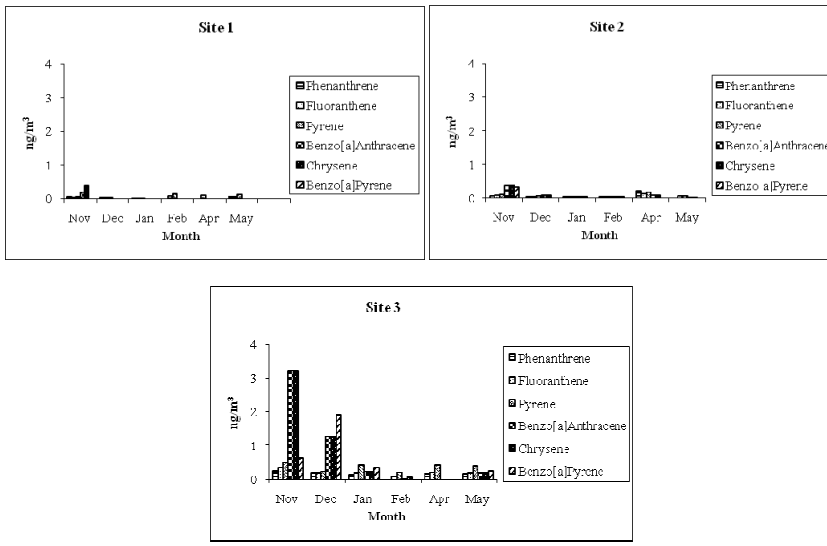

Figure 1: PAH concentrations in ng/m^3 of the three sites.

The highest concentrations of PAHs were at site 3, where benzo[a]anthracene (3.23 ng/m^3) and chrysene (3.23 ng/m^3) were predominant in November, and benzo[a]pyrene (1.90 ng/m^3) was predominant in December.

3.3 Analysis of nitro-polycyclic aromatic hydrocarbons (nitro-PAHs)

Figure 2 shows the nitro-PAH concentrations, in ng/m^3, at the three sites.

Site 3 showed the highest concentrations of nitro-PAHs. The predominant nitro-PAHs at site 3 were: (November) nitro-pyrene (2.52 ng/m^3), nitro-phenanthrene (2.30 ng/m^3), nitro-acenaphthene (2.10 ng/m^3), nitro-acenaphthylene (2.07 ng/m^3), nitro-anthracene (2.06 ng/m^3) and nitro-fluorene (1.97 ng/m^3);(December) nitro-anthracene (1.56 ng/m^3), nitro-phenanthrene (1.46 ng/m^3) and nitro-fluorene (1.39 ng/m^3); (January) nitro-benzo[k]fluoranthene (2.28 ng/m^3), nitro-acenaphthylene (2.20 ng/m^3), nitro-fluorene (1.87 ng/m^3), nitro-phenanthrene (1.86 ng/m^3) and nitro-anthracene (1.71 ng/m^3);(February) nitro-benzo[a]pyrene (1.86 ng/m^3), nitro-benzo[b]fluoranthene (1.55 ng/m^3) and nitro-chrysene (1.48 ng/m^3); (April) nitro-benzo[b]fluoranthene (2.08 ng/m^3) and nitro-phenanthrene (1.93 ng/m^3); (May) nitro-phenanthrene (1.73 ng/m^3), nitro-fluorene (1.93 ng/m^3) and nitro-anthracene (1.34 ng/m^3).

The predominant nitro-PAHs at site 2 were: (November, January and February) nitro-chrysene (0.57–0.84 ng/m^3) and nitro-benzo[b]fluoranthene

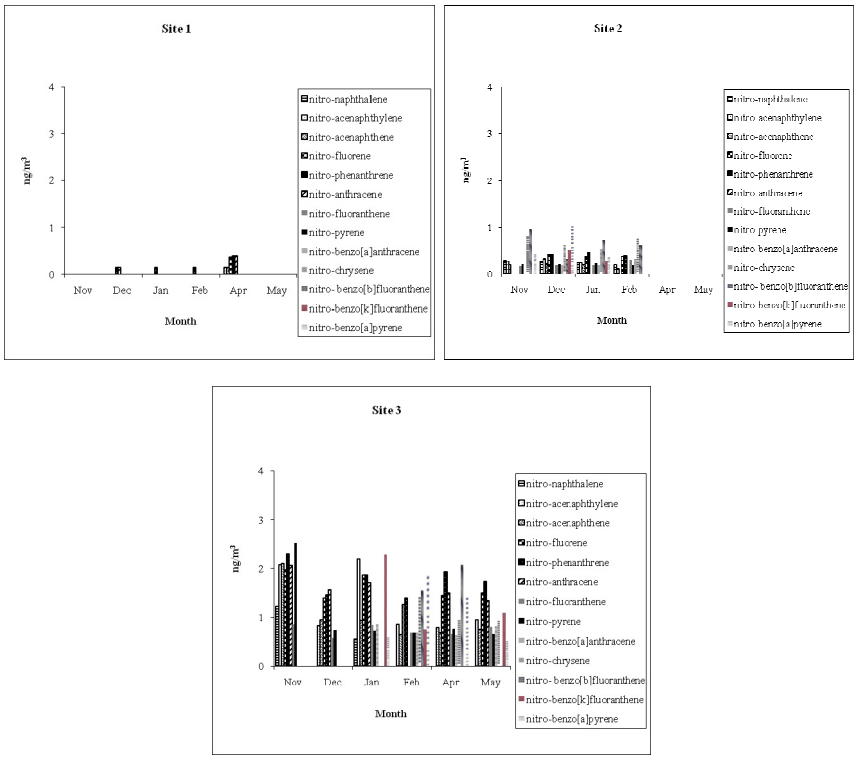

Figure 2: Nitro-PAH concentrations in ng/m^3 of the three sites.

(0.61–0.96 ng/m^3); (December) nitro-chrysene (0.65 ng/m^3) and nitro-benzo[a]pyrene (1.04 ng/m^3). No nitro-PAHs were detected at site 2 in April or May.

The predominant nitro-PAHs at site 1 were: (December) nitro-phenanthrene (0.14 ng/m^3) and nitro-anthracene (0.15 ng/m^3); (January) nitro-phenanthrene (0.14 ng/m^3); (February) nitro-phenanthrene (0.14 ng/m^3); (April) nitro-phenanthrene (0.39 ng/m^3), nitro-anthracene (0.39 ng/m^3) and nitro-fluorene (0.37 ng/m^3). No nitro-PAHs were detected at site 1 in November or May.

3.4 Salmonella/microsome assay

Table 2 shows the mutagenicity data for the organic extracts from airborne particulate matter in rev/m^3 during period II. Cytotoxic effects were not detected for any of the samples analyzed.

Mutagenic frameshift responses in the presence of metabolic activation were detected only at site 3 (November, December and May) (Table 2).

Mutagenic frameshift responses in the absence of metabolic activation were detected at site 1 (November, December and April), site 2 (December, January, February, April and May) and site 3 (November and April) (Table 2).

Table 2: Induced mutagenic by airborne particulate matter organic extracts (rev/m^3).

Site	Month	TA98		YG1021		YG1024	
		-S9	+S9	-S9	+S9	-S9	+S9
1	Nov	9.10 ± 1.30	n.d.[a]	9.10 ± 1.50	7.00 ± 1.80	n.d.[a]	n.d.[a]
	Dec	1.50 ± 0.40	n.d.[a]	3.10 ± 0.40	1.20 ± 0.20	n.d.[a]	n.d.[a]
	Jan	n.d.[a]	n.d.[a]	n.d.[a]	n.d.[a]	n.d.[a]	n.d.[a]
	Feb	n.d.[a]	n.d.[a]	n.d.[a]	n.d.[a]	n.d.[a]	n.d.[a]
	Apr	0.10 ± 0.01	n.d.[a]	0.20 ± 0.10	0.80 ± 0.10	n.d.[a]	n.d.[a]
	May	n.d.[a]	n.d.[a]	n.d.[a]	n.d.[a]	n.d.[a]	n.d.[a]
2	Nov	n.d.[a]	n.d.[a]	n.d.[a]	n.d.[a]	19.20 ± 1.70	4.70 ± 0.60
	Dec	2.10 ± 0.30	n.d.[a]	4.10 ± 1.00	3.50 ± 0.90	13.60 ± 1.90	4.80 ± 0.80
	Jan	4.40 ± 0.70	n.d.[a]	2.80 ± 0.40	n.d.[a]	16.00 ± 1.40	3.40 ± 0.60
	Feb	4.60 ± 1.00	n.d.[a]	n.d.[a]	n.d.[a]	3.90 ± 1.10	n.d.[a]
	Apr	4.80 ± 0.60	n.d.[a]	2.50 ± 0.20	0.30 ± 0.10	2.80 ± 0.60	2.20 ± 0.40
	May	18.80 ± 3.30	n.d.[a]	21.70 ± 3.40	16.60 ± 2.60	30.90 ± 1.80	10.60 ± 1.10
3	Nov	44.20 ± 24.30	49.80± 7.10	45.40± 13.60	34.70± 7.70	83.00± 5.00	8.60 ± 3.90
	Dec	n.d.[a]	19.20± 4.90	n.d.[a]	n.d.[a]	51.70± 5.40	n.d.[a]
	Jan	n.d.[a]	n.d.[a]	20.30± 4.90	n.d.[a]	21.90± 6.00	n.d.[a]
	Feb	n.d.[a]	n.d.[a]	2.50 ± 0.50	n.d.[a]	n.d.[a]	n.d.[a]
	Apr	17.00 ± 2.40	n.d.[a]	12.30 ± 2.40	n.d.[a]	8.20 ± 1.30	n.d.[a]
	May	n.d.[a]	5.80 ± 1.30	n.d.[a]	n.d.[a]	n.d.[a]	n.d.[a]

1 – UERJ; 2 – Avenida Brasil; 3 – Rebouças tunnel. n.d. [a] – not detected. Negative Control: DMSO for the mutagenicity assay without S9 mix were: TA98, (18±8); YG1021, (28±4); YG1024, (17±2). For the mutagenicity assay with S9 mix were: TA98, (38±7); YG1021, (32±8); YG1024, (20±7). Positive Controls for the mutagenicity assay without S9 mix were: 4-nitroquinoline oxide (0.5 µg/plate) for TA98, (42±8); YG1021, (60±2); YG1024, (101±37). For the mutagenicity assay with S9 mix were: 2-aminofluorene (1 µg/plate) for TA98, (134 ± 4); YG1021, (687±61); YG1024, (67±13).

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The presence of nitroarenes was detected at site 1 in November, December and April; at site 2 in December and May; and at site 3 in November, January and February. The presence of dinitroarenes and hydroxylamines was detected at site 2 in November, December, January and May, and at site 3 in November, December and January.

4 Discussion

The average PM2.5 concentrations at all three sites were higher during period II than recommended by the WHO (site 3: 54–141 $\mu g/m^3$; site 2: 27–38 $\mu g/m^3$; site 1: 25 and 34 $\mu g/m^3$), which recommends a daily mean of up to 25 $\mu g/m^3$[11]. Vehicle emissions are a major source of particles, especially in urban areas [20–22]. The fleet of vehicles in Rio de Janeiro has tripled in the last twenty years, and the high values of PM2.5 can be related to this fact [10]. Sites 2 and 3 are areas of the city that have heavy traffic, and they are the sites that yielded the highest average levels of PM2.5. Site 3 is a tunnel, and besides the heavy traffic it also lacks adequate ventilation, which could hamper the dispersion of pollutants.

High levels of PM2.5 were also detected during period I (site 3: 94–132 $\mu g/m^3$; site 2: 26–60 $\mu g/m^3$; site 1: 30–36 $\mu g/m^3$) at these same sites [9, 10]. When comparing the two periods (July to October 2010 and November 2010 to May 2011), we observed higher PM2.5 values in period I. A study conducted from August 2010 to March 2011 in Porto Alegre, Brazil, detected high concentrations of PM2.5 in August [4]. The increased values of PM at this time of year can be related to the winter weather conditions. The reduction in PM2.5 values at site 3 during January and February 2011 (period II) may be related to the summer school vacations, during which time there are fewer vehicles on the roads.

PAHs are formed from the incomplete combustion of organic matter and may be introduced into the environment from several sources. Their main source in urban areas, according to Reisen and Arey [23], is exhaust gases from internal combustion engines, especially diesel [14]. Owing to their utility as tracers, it is essential to document their atmospheric abundance to identify the sources in different environments [24].

The highest PAH concentrations detected at site 3 are caused by vehicular emissions and restricted ventilation. These PAHs are classified according to their carcinogenicity by the International Agency for Research on Cancer [25]: benzo[a]pyrene is in Group 1 (carcinogenic to humans), and benzo[a]anthracene and chrysene are in Group 2B (possibly carcinogenic to humans).

Although site 2 has heavy traffic, it is in an area that has many dispersion factors, such as wind and rain, which could explain why its PAH levels are lower than at site 3. When comparing the two periods, we observed similar PAH values at sites 1 and 2 [10]. However, in November (during Period II), only benzo[a]anthracene and chrysene were detected at site 1, and only benzo[a]pyrene was detected at site 2. Periods with high temperatures and photochemical decomposition are propitious for PAHs dispersal. Several studies

that monitor levels of PAHs have detected a reduction in these pollutants during the summer [26–28].

Site 3 had the highest values for benzo[a]anthracene, chrysene and benzo[a]pyrene during period II (November and December). The increase in these PAHs may be related to gasoline and diesel vehicular emissions [29, 30].

Nitro-PAHs present in the atmosphere originate from primary sources, such as vehicle emissions, especially from diesel-fueled vehicles [31–33]. In addition, nitro-PAHs are also formed in the atmosphere via a reaction of their parent PAHs initiated by hydroxyl (OH) radicals during the day and by nitrate (NO_3) radicals (in the presence of NO_x) during the night [33–35] and/or the heterogeneous gas–particle interaction of the parent PAHs adsorbed onto particles with nitrating agents [36]. Nitro-PAHs have 2.10^5 times the mutagenic and ten times the carcinogenic potential of PAHs [33, 37].

In period I no nitro-PAH analysis was performed, which prevents a comparison between the two periods. Site 3 showed the highest concentrations of nitro-PAHs. This result may be related to vehicular emissions and inadequate ventilation. The nitro-PAH levels detected at site 3 were up to 21 times higher than in other tunnels: Allegheny Mountain (0.12 ng/m^3 1-nitropyrene) [38]; Baltimore Harbour (0.34 ng/m^3 1-nitropyrene and 0.29 ng/m^3 9-nitroanthracene) [39] and Queensway (0.56 ng/m^3 1-nitropyrene and 0.36 ng/m^3 9-nitroanthracene) [40].

Meanwhile, site 1 was found to have the lowest nitro-PAH values. This may be related to the lower automotive emissions than at the other two sites under study. The highest nitro-PAH values were at site 2, especially during the hottest months of the year. Similar nitro-PAH values were detected during the summer in the metropolitan area of Porto Alegre [33]. These results may be related to the interaction between PAHs and nitro compounds in photochemical smog.

All strains detected higher rev/m^3 in period I [9, 10] than in period II at all three sites. There was less rainfall in period I than in period II. This seasonal characteristic could lead to a concentration of pollutants and thereby increased values of rev/m^3. Increased mutagenic responses during the winter have been detected in several studies that evaluate the mutagenicity of PM seasonally [2, 4, 26]. Decreased levels of rev/m^3 were detected mainly in the rainy months (December 2010 to February 2011 – period II). Rain cleanses some particles from the atmosphere, but this does not mean that there is no further risk of exposure to contaminants associated with the particles that remain in the environment [2, 41, 42].

For period I, mutagenic frameshift responses in the presence of metabolic activation were detected at all three sites [9, 10]. However for period II mutagenic frameshift responses in the presence of metabolic activation were detected only at site 3. These results can be attributed to the presence of promutagens such as PAH, resulting from intense vehicular emissions and inadequate ventilation in this tunnel.

Mutagenic frameshift responses in the absence of metabolic activation were detected at all the sites during periods I [9] and II. These results could indicate the predominance of direct-acting frameshift activity in the airborne particulate

material. Several authors have recognized the contribution of nitrocompounds to direct frameshift mutagenicity in urban atmospheric samples, associating this capacity with the presence of PAH derivatives, such as mono- or dinitro PAHs [3, 4, 12]. The contribution of nitrocompounds to direct mutagenic activity was investigated through the *Salmonella/microsome* assay with specific strains YG1021 (pYG216) and YG1024 (pYG219), which over express highly active enzymes with a high sensitivity to nitrocompounds such as nitroarenes or dinitrorenes, hydroxylamines and aromatic amines [18]. In period I, nitroarenes and dinitroarenes were detected at all three sites [9, 10]. During period II, the presence of nitroarenes was also detected at all the sites, but dinitroarenes were only detected at sites 2 and 3. Nitroarenes and dinitroarenes result from direct emissions of diesel combustion and can be produced by atmospheric reactions of PAH with gaseous copollutants found in photochemical smog [43, 44]. Studies performed in the urban region of Porto Alegre detected a similar response during the hot season, when there is marked mutagenic activity due to the presence of mono and dinitroarene compounds in different sizes of atmospheric particles such as PTS, PM10 and PM2.5 [4, 45].

5 Conclusion

In conclusion, air quality in Rio de Janeiro is worsening as traffic becomes heavier. The data generated in this study show that in certain periods of the year, different levels of PAHs and nitroderivatives probably contribute to the airborne mutagenicity detected at different sites of Rio de Janeiro. However, less variation in the levels of PAHs and nitroderivatives was found in Rio de Janeiro than has been reported in other places where winter temperatures are lower. This may be attributed to the fact that the city of Rio de Janeiro does not have wide temperature fluctuations during the year.

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