Occurrence of polycyclic aromatic hydrocarbons derivatives and mutagenicity study in extracts of PM$_{10}$ collected in São Paulo, Brazil

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Ocorrência de derivados de hidrocarbonetos policíclicos aromáticos e estudo da sua mutagenicidade em extratos de MP$_{10}$ coletado em São Paulo, Brasil

As frações nitro-HPA e oxi-HPA de extratos orgânicos de MP$_{10}$ coletados na cidade de São Paulo, Brasil, (inverno, 2004) foram quimicamente e toxicologicamente analisadas. Alguns nitro-HPA emitidos por exaustão veicular foram quantificados. O 3-nitrofluoranteno, provavelmente co-eluído com o 2-nitrofluoranteno, um poluente secundário, apresentou níveis mais elevados (média: 0,16 ng m$^{-3}$; concentração máxima: 0,26 ng m$^{-3}$) que o 1-nitropireno (média: 0,031 ng m$^{-3}$; concentração máxima: 0,054 ng m$^{-3}$). O 6-nitrocreseno não foi encontrado em níveis detectáveis. Oxi-HPA não foram detectados provavelmente porque esses compostos estavam presentes em concentrações muito baixas. Ambas as frações, nitro-HPA e oxi-HPA apresentaram respostas mutagênicas semelhantes, do tipo deslocamento no quadro de leitura, com potencial maior na ausência de S9 quando comparado com a resposta na presença de S9, indicando a prevalência de mutágenos diretos para o teste Salmonella/microsoma. As respostas observadas para a linhagem YG1041 (valores até 1200 revertentes μg$^{-1}$) foram significativamente maiores que para a TA98 (valores até 28 revertentes μg$^{-1}$) sugerindo a presença de compostos nitroaromáticos. No passado, a presença de compostos altamente mutagênicos nas frações nitro-HPA e compostos oxigenados contendo radical NO$_2$ na fração oxi-HPA foi evidenciada.

Palavras-chave: poluição do ar urbano, material particulado, nitro-HPA and oxi-HPA, mutagenicidade

INTRODUCTION

Although many classes of potentially genotoxic compounds have been studied in ambient air samples, mutagenic compound identification in urban air particles is still limited due to the complexity of the extractable organic matter. Fractionation procedures to separate the organic extract into substantially less complex fractions are used to facilitate isolation and identification of mutagenic compounds (1-4).

Polycyclic aromatic hydrocarbons (PAHs) play a significant role on the mutagenic activity of airborne particles. However, approximately 50-60% of the mutagenicity on atmospheric particulate matter is associated with classes of compounds that are more polar than PAHs. PAH derivatives normally present higher mutagenic activity than their PAH parents (3-7). Nitro-PAH and oxy-PAH are generally the responsible compounds for the high mutagenicity found in more polar compounds containing fractions (8).

Nitro- and oxy-PAHs have been detected in ambient air particles at much lower concentrations than their parent PAHs (3,9-14). In semi-rural and urban atmospheres, detectable levels of nitro-PAH and oxy-PAH have been found (14). They are formed from parent PAH, either by radical or nitration/oxidation reactions, or they are released directly into the atmosphere by incomplete combustion of carbonaceous materials (15-21). They have been detected in the extracts of diesel and gasoline emissions, fly ash
Sampling procedure

Samples were collected with a Hi-vol PM_{10} sampler using quartz fiber filters, 254 x 203 mm (Pallflex). Twenty-four hour samples were collected from 8:00 a.m. Before sampling, filters were pre-cleaned by heating in oven at 800°C for 8 h. After sampling, filters were packed in aluminum foil and stored in freezer at -20°C until they were weighted and extracted. Sampling was performed on consecutive August days from 3rd to 12th in the winter, 2004 (samples 1 to 10) and one sample was collected on July, 22nd in the winter, 2003 (sample 11); total samples taken = 11, total samples chemically analyzed = 11 and total samples toxicologically analyzed = 6. Because of the relatively high cost of the Salmonella/microsome microsuspension assay, only some representative samples were toxicologically analyzed.

Sampling site characteristics

São Paulo is located in the southeastern part of the Brazil. The metropolitan area of São Paulo City is the largest industrialized region in Latin America. It is characterized by a subtropical climate. It has an area of 8051 km² with a population of over 19 million inhabitants. The region has about 2000 industries with high pollution potential and a fleet of approximately 8.4 million registered vehicles (7.0 million Otto-cycle vehicles, 460 thousand diesel vehicles, and 1020 million motorcycles), which corresponds to 1/5th of the national fleet (36,37).

In this work, a site within on the main campus of the University of São Paulo (USP), located in the southwestern area of the São Paulo City, Brazil, was chosen for sampling. The sampling site that can be considered potentially impacted by many different types of sources is ~ 2 Km from a major highway with heavy vehicle traffic fueled by gasohol, diesel, and ethanol. At this site, sampler was placed in an open area on the roof of the Institute of Astronomy, Geophysics and Atmospheric Sciences (IAG), ~ 20 m above ground level. During the sampling period, solar radiation ranged from 105 to 318 cal cm⁻² and average temperature was of 17°C. In the sampling site, on August, 3rd to 12th, 2004 and July, 22nd, 2003, O₃ and NOₓ were recorded (O₃ = 10.8 to 16.6 ppb; NOₓ = 13.7 to 26.9 ppb and O₃ = 17.4 ppb; NOₓ = 26.1 ppb).

Extraction and fractionation

Filters were weighted before and after sampling for mass determination of PM_{10}. Fresh sample filters were extracted by a Soxhlet apparatus with dichloromethane, DCM (Mallinckrodt-HPLC grade, 99.9%) for 20 h. The Soxhlet reflux cycle time was approximately 15 min. Each extract was concentrated using a rotary evaporator at 40°C followed by evaporation with a gentle stream of nitrogen gas until almost dryness. Then, the residue obtained was resuspended in 50 µL of DCM and filtered by PTFE-membrane, 0.45 µm from Millipore prior to HPLC injection. The organic extract was separated on silica normal phase column (type ‘Nucleosil 100-10’; length, 250 mm; id., 4.6 mm; provided by Macherey-Nagel) using a solvent gradient from n-hexane (Mallinckrodt-HPLC grade, 85%) to DCM; the first 10 min was used n-hexane (100%) and after that a DCM gradient was used until reach 100% of DCM. An adapted elution procedure of that described by Ciccioli et al. (38) was used.
Four fractions were collected according to the following elution times: the non polar fraction from 0.0 until 7.0 min (alkanes fraction), the moderately polar fraction from 7.0 to 17.5 min (PAH fraction), the polar fraction from 17.5 to 28.0 min (nitro-PAH fraction) and the very polar fraction from 28.0 to 45.0 min (oxy-PAH fraction). A standard mixture containing alkane, PAH, nitro-PAH and oxy-PAH was used to check the elution times of the fractions. Alkane and PAH fractions were not focused in this work. All fractions were kept in the freezer at -20°C until they were weighted and analyzed.

Chemical analysis

Nitro-PAH and oxy-PAH fractions were analyzed by a GCMS-QP5000 Shimadzu gas chromatograph coupled to a mass spectrometer equipped with a DB-1 fused-silica capillary column (30 m x 0.25 µm film thickness). The temperature program used is described in detail by Ciccioli et al., (38). The splitless mode was used for sample introduction.

Nitro-PAH were quantified by the external standard method. Standards (Aldrich) of 3-nitrofluoranthene (3-NFlt), 1-nitropyrene (1-NPyr) and 6-nitrochrysene (6-NChr) available in our laboratory were used. The single ion monitoring (SIM) detection method was used for quantification. The specific ions monitored for 3-NFlt and 1-NPyr were m/z 247; m/z 217; m/z 201; m/z 200 and m/z 189, and for 6-NChr were m/z 273; m/z 243 and m/z 226. Due to small differences in GC retention times for 2- and 3-nitrofluoranthene, these isomers coelute on DB-1 fused-silica capillary 100% dimethyl polysiloxane column used (6,39). In this work, the chromatographic peak corresponding to the 3-NFlt is taken into as 3-NFlt + 2-NFlt isomers. For the best separation of the nitrofluoranthene isomers, a 50%-phenyl-methylpolysiloxane column (DB-17) might be used (40). The detection limits of the nitro-PAH compounds studied are presented in Table 1. Standards of oxy-PAH were not available in our laboratory, except the 3-nitrobenzanthrone (3-NBA).

Recovery tests of 3-NFlt, 1-NPyr and 6-NChr were previously performed and values above of 77.4% were obtained (Table 1). Similar result was found for 1-NPyr in Standard Reference Material (SRM) 1650, Diesel Particulate Matter of National Institute of Standards and Technology (NIST).

Salmonella/microsome microsuspension assay

Nitro-PAH and oxy-PAH fractions of some PM_{10} samples (samples 1, 2, 4, 8 and 9) were assayed for mutagenicity using a microsuspension assay (41) with Salmonella typhimurium, strains TA98 (hisD5302, rfa, Abio, AuvRr, pKM101) (42) and YG1041 (a derivative of the TA98, able to produce high levels of nitroreductase and O-acetyltransferase) (43) in the presence and absence of exogenous metabolic activation system (± S9). Overnight cultures of strains (around 10^9 cells mL^{-1}) 5-fold concentrated by centrifugation (10,000 g at 4°C for 10 min) were resuspended into 0.015 M sodium phosphate buffer. 50 µL of cell suspension, 50 µL of 0.015 M sodium phosphate buffer or S9 mix, and 5 µL of the sample were added to a tube and incubated at 37°C for 90 min without shaking. After incubation, 2 mL of molten agar was added, and the mixture was poured onto a minimal agar plate. Colonies were counted after 6 h of incubation at 37°C using an automatic colony counter. The metabolic activation was provided by Aroclor 1254 induced Sprague Dawley rat liver S9 mix (MolTox, Boone, NC), which was prepared at a concentration of 4% v/v.

For TA98 assay, 0.125 µg per plate of 4-nitroquinoline-oxide (4NQO) (Acros) and 0.625 µg per plate of 2-aminoanthracene (2AA) (Sigma-Aldrich) both dissolved in dimethyl sulfoxide (DMSO) were used as positive controls. For YG1041 assay, 5 µg per plate of 4-nitro-o-phenylenediamine (4NOP) (ICN Biomedicals Inc) and 0.0312 µg per plate of 2AA were used as positive controls. Each fraction was solvent exchanged to DMSO using a gentle stream of pure nitrogen gas. The doses tested varied from 0.01 to 30 µg of the organic extract of each fraction per plate depending on the strain and the condition tested. Each sample was assayed using two replica plates per dose, except for the negative control that was tested in five replicates. In order to verify the contribution of 1-NPyr to the mutagenicity of nitro-PAH fractions, six doses of 0.1 to 30 ng of the compound per plate were tested for TA98 strain, without S9, in two replica plates. For TA98, with S9, doses of 10 to 3000 ng of the compound per plate were requested due to the variability in the responses. For YG1041 strain, without and with S9, doses of 0.01 to 30 ng per plate were tested as nitro-PAH compounds present high sensitivity for these mutagenicity assays (see Table 2). The calculated potency in rev ng^{-1} of the individual 1-NPyr was: 126 rev ng^{-1} for TA98 strain, without S9 and 1.2 rev ng^{-1} for TA98 strain, with S9; 6900 rev ng^{-1} for YG1041 strain, without S9 and 234 rev ng^{-1} for YG1041 strain, with S9.

Data were analyzed with the Salanal computer program using the Bernstein model (44). Positive results were considered when a significant difference among the tested doses and the negative control (ANOVA; p<0.05) and a significant dose response was observed (p<0.05). Results for ambient air samples and 1-NPyr were expressed as the number of revertants per mass unit of extract (revertants µg^{-1}).

Table 1. Detection limit of the nitro-PAH by GC-MS and recovery efficiency.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Detection limit (µg mL^{-1})</th>
<th>Recovery (%)</th>
<th>n = 20</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-NFlt</td>
<td>40.00</td>
<td>84.4</td>
<td></td>
</tr>
<tr>
<td>1-NPyr</td>
<td>31.00</td>
<td>77.4</td>
<td></td>
</tr>
<tr>
<td>6-NChr</td>
<td>17.00</td>
<td>83.9</td>
<td></td>
</tr>
<tr>
<td>1-NPyr*</td>
<td></td>
<td>75.5</td>
<td></td>
</tr>
</tbody>
</table>

* SRM 1650 sample.
Gravimetric determination of the fractions

Nitro-PAH and oxy-PAH fractions were submitted to gravimetric determination. Each fraction was removed from freezer, brought to room temperature and sonicated for 20 to 30 s in a sonicator bath. The fraction volume was checked against a mark on the vial to avoid any solvent loss. 50 µL aliquots were weighted in a thermo-balance TGA-50 (Shimadzu) in a tared platinum weighing pan. The solvent was evaporated to dryness using nitrogen at flux of 10 mL min⁻¹ and temperature raising at 2°C min⁻¹ until 40°C.

RESULTS AND DISCUSSION

As described previously, identification of mutagenic compounds present in organic extracts of atmospheric particulate matter is difficult due to matrix complexity. Extracts from PM₁₀ samples collected in the atmosphere of São Paulo City were normal-phase high performance liquid chromatography (HPLC) fractionated and two containing nitro-PAH and oxy-PAH fractions were chemically analyzed. Some nitro-PAH compounds were identified and quantified and some oxy-PAH compounds were only identified. Nitro-PAH and oxy-PAH fractions of some samples were toxicologically analyzed.

Nitro-PAH compounds

Results of concentration of 3-NFilt + 2-NFilt and 1-NPyr in the containing nitro-PAH organic fractions are presented in Table 3. The 3-NFilt, as previously described, was probably eluted with 2-NFilt. The 3-NFilt + 2-NFilt isomers presented higher levels (average: 0.16 ng m⁻³; maximum concentration: 0.26 ng m⁻³ and 18% of samples < LOD) than 1-NPyr (average: 0.031 ng m⁻³; maximum concentration: 0.054 ng m⁻³ and 45 % of samples < LOD) and no detectable amount of 6-NChr was found in all samples. The 3-NFilt, 1-NPyr, as well as 6-NChr compounds are known as primary pollutants from diesel motor exhaust emissions (3,13,14,45). It is interesting to point out that 1-NPyr concentrations in our samples were quite similar to those found in samples collected at the same sampling site in a previous study (46).

Table 3. Concentration of 1-NPyr, 3-NFilt + 2- NFilt and PM₁₀ (winter, 2004 – São Paulo City).

<table>
<thead>
<tr>
<th>Sample</th>
<th>3-NFilt + 2-NFilt (ng m⁻³)</th>
<th>1-NPyr (ng m⁻³)</th>
<th>PM₁₀ (mg m⁻³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.170</td>
<td>0.016</td>
<td>0.136</td>
</tr>
<tr>
<td>2</td>
<td>0.230</td>
<td>0.029</td>
<td>0.065</td>
</tr>
<tr>
<td>3</td>
<td>0.227</td>
<td>0.045</td>
<td>0.095</td>
</tr>
<tr>
<td>4</td>
<td>0.228</td>
<td>0.054</td>
<td>0.073</td>
</tr>
<tr>
<td>5</td>
<td>0.100</td>
<td>&lt; LOD</td>
<td>0.046</td>
</tr>
<tr>
<td>6</td>
<td>&lt; LOD</td>
<td>&lt; LOD</td>
<td>0.032</td>
</tr>
<tr>
<td>7</td>
<td>0.036</td>
<td>&lt; LOD</td>
<td>0.026</td>
</tr>
<tr>
<td>8</td>
<td>&lt; LOD</td>
<td>&lt; LOD</td>
<td>0.047</td>
</tr>
<tr>
<td>9</td>
<td>0.116</td>
<td>&lt; LOD</td>
<td>0.056</td>
</tr>
<tr>
<td>10</td>
<td>0.110</td>
<td>0.018</td>
<td>0.036</td>
</tr>
</tbody>
</table>

LOD = limit of detection.
Higher levels of 2-NFlt compared to the 3-NFlt have been reported in the literature (14). Whereas 3-NFlt has been detected in diesel exhaust, 2-NFlt has been found to be one of the most abundant nitro-PAHs in ambient particulate matter in most locations not associated with vehicle emissions. The 2-NFlt isomer is formed from the parent fluoranthene by gas-phase hydroxyl radical- or nitrate radical-initiated reactions in the atmosphere (6).

By comparing published results on 1-NPy, it is possible to observe that the levels found in our study are comparable to those found in Claremont, USA, 1989 (0.003 - 0.06 ng m\(^{-3}\)) (47); São Paulo, Brazil, 1995 (< 0.016 ng m\(^{-3}\)) (25); Rome, Italy, 1999 (0.12 ng m\(^{-3}\)) (27); Athens, Greece, 2000 (0.02 - 0.18 ng m\(^{-3}\)) (28) and São Paulo, Brazil, 2002 and 2003 (average: 0.017 and 0.031 ng m\(^{-3}\), respectively) (46). The results of 3-NFlt + 2-NFlt are comparable to those observed in Rochester and Boston, USA, 1995 (0.038 and 0.090 ng m\(^{-3}\), respectively) (39).

Concentrations of 2-NFlt have been found to be greater than those of 1-NPy and different values for 2-NFlt/1-NPy ratios in different urban regions have been observed; for example, 8.7 in São Paulo (25), 3.1 in Rome (26), and 2.3 in Athens (28). In our study, a relatively high ratio of 3-NFlt + 2-NFlt/1-NPy was obtained (average = 7.1; range = 4.2 to 10.6). This result shows that the 2-NFlt isomer may be present in the airborne particles collected in São Paulo, as it has been found in airborne particulate at higher concentrations than the 3-NFlt.

In this study, the nitro-PAH compounds quantified were probably emitted from vehicular motor exhaust, in particular, diesel exhaust. The 2-NFlt, the nitro-PAH compound supposedly present in the airborne particles collected is known as a secondary pollutant formed in the atmosphere (6).

**Oxy-PAH compounds**

Many PAHs undergo photochemical oxidation while adsorbed onto atmospheric particles. Oxy-PAH compounds, such as 9,10-anthracenedione (anthraquinone), 7H-benzo(Le)anthracenedione (benzanthrone), 9H-fluorene-9-one and 3-nitrobenzanthrone have been found in urban atmosphere at low concentrations (14). Much higher levels of 9,10-anthracenedione and 9H-fluorene-9-one, however, were observed in tunnel air (23).

The oxy-PAH compounds in wintertime ambient PM\(_{10}\) air samples collected in São Paulo City (August, 3-12, 2004) were not detected under analytical conditions used. They might be present at concentrations below the detection limit of the analytical method. Comparing 2003 and 2004 winter data of wind speed recorded by Meteorological Station of the IAG-USP and results of the gravimetric determination of PM\(_{10}\), it is possible to observe that a pollution dispersion

![Figure 1](image1.png)

*Figure 1. Profiles of wind speed in São Paulo area in winter, 2003 and 2004 (data obtained from IAG-USP).*

![Figure 2](image2.png)

*Figure 2. Comparison of PM\(_{10}\) concentrations in winter, 2003 and 2004 (data obtained from IAG-USP).*
episode (Figures 1 and 2) occurred in August, 2004. It might explain the decrease of the PM$_{10}$ concentration and eventually the oxy-PAH amount associated to the particulate matter in this period. It is interesting to mention here that some oxy-PAH compounds, such as 9,10-anthracenedione, 5H-phenanthro(4,5-bcd)pyrane-5-one and benzanthrone, as well as dehydroabietic acid, a tracer for wood smoke (48), were detected in one wintertime sample (sample 11, not shown in Table 3) collected at the same sampling site in the year before (July, 22, 2003). This fact shows that if analytical protocol modifications, such as the use of on-column injection for sample introduction into the gas chromatograph (49), had been done, an increase of the sensitivity could have been reached and oxy-PAH compounds might have been eventually detected.

Although the 3-NBA, a nitrated polycyclic ketone known as a highly potent direct-acting mutagen, had not been detected in our samples, it may eventually be present. The 3-NBA concentration probably was below the detection limit of the analytical method (0.50 ng µL$^{-1}$). HPLC analysis using the 3-NBA standard showed that this bi-functional PAH is in the more polar fraction, the oxy-PAH fraction. Several authors reported that the reaction of the benzanthrone, an PAH ketone emitted directly from combustion engines, with NO$_x$ in the atmosphere could produce the 3-NBA (26,50,51).

In a recent study, however, it was shown that the 3-NBA is emitted directly from vehicular motor exhaust whereas the 2-NBA is formed in the atmosphere (38).

**Inhalable airborne particulate matter (PM$_{10}$)**

The concentration of PM$_{10}$ in the air samples collected during the winter season, 2004, which ranged from 26.2 µg m$^{-3}$ to 136.0 µg m$^{-3}$, are presented in Table 3. They revealed that a half of the samples presented PM$_{10}$ values above the recommended World Health Organization and Brazilian standards (50 and 150 µg m$^{-3}$), respectively.

By examining PM$_{10}$, 1-NPyr and 3-NFlt + 2-NFlt concentrations, it was possible to observe that PM$_{10}$ is correlated to the 1-NPyr ($r = 0.49$) and the 3-NFlt + 2-NFlt ($r = 0.65$). These results suggest that PM$_{10}$ should come from similar or identical emission sources to those of nitro-PAHs detected.

**Mutagenicity of the fractions**

All nitro- and oxy-PAH fractions of PM$_{10}$ samples assayed presented mutagenic activities with the *Salmonella typhimurium* TA98 and YG1041 strains in the presence and absence of exogenous metabolic activation system, ± S9 (Table 4). Both nitro- and oxy-PAH fractions have showed a similar frameshift mutagenic response with a higher potency in the absence of S9 compared to that in the presence of S9, indicating the prevalence of direct-acting compounds for the *Salmonella/microsome* assay. The responses observed for the YG1041 (up to 1200 revertants µg$^{-1}$) were significantly higher than for the TA98 (up to 28 revertants µg$^{-1}$) suggesting the contribution of nitroaromatic compounds to the mutagenic activity observed. Due to the high levels of nitroreductase present in the YG1041 strain, the NO$_2$ derivatives are reduced to hydroxylamines which are mutagenic compounds. Those hydroxylamines can be further acetylated by the acetyltransferase, also abundant in the YG1041, generating compounds with even higher mutagenic activity.

In order to know the relative contribution of the 1-NPyr to the mutagenicity of nitro-PAH fractions, a dose response test of the 1-NPyr was done under the same conditions for those used for nitro- and oxy-PAH fractions. For TA98 strain, without S9, the 1-NPyr compound contributed from 18 to 55 %, and with S9, from 0.4 to 1.0%. For YG1041 strain, without S9 (Figure 3a) contributed from 23 to 86% and with S9, from 5.5 to 9.4% (Figure 3b).

The 2-NFlt and 3-NFlt isomers could partially account for the mutagenicity detected but their individual contribution could not be determined in the current work because the co-elution problem previously discussed. The mutagenicity of nitro-PAH fractions is related to the presence of the 1-NPyr but it does not account for the whole mutagenic activity. On the other hand, compound like the 3-NBA, that is a highly potent mutagenic agent, should probably contribute to the mutagenicity of these fractions along with others. Recently, Umbuzeiro *et al.* (8) showed that although

**Table 4. Mutagenicity of nitro- and oxy-PAH fractions by using microsuspension assay with *Salmonella typhimurium* strains TA98 and YG1041 with and without S9.**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Nitro-PAH fraction</th>
<th>Oxy-PAH fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TA98</td>
<td>YG1041</td>
</tr>
<tr>
<td></td>
<td>-S9</td>
<td>+S9</td>
</tr>
<tr>
<td>1</td>
<td>2.4</td>
<td>1.1</td>
</tr>
<tr>
<td>2</td>
<td>21.0</td>
<td>9.6</td>
</tr>
<tr>
<td>4</td>
<td>10.0</td>
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<tr>
<td>8</td>
<td>10.0</td>
<td>2.4</td>
</tr>
<tr>
<td>9</td>
<td>4.5</td>
<td>1.5</td>
</tr>
</tbody>
</table>
the 3-NBA has not been detected, the oxy-PAH fractions of atmospheric particulate matter from São Paulo contain potent compounds able to produce DNA adducts under reductive conditions.

Comparison between mutagenicity and mass of the fractions

Results of mutagenicity (YG1041, - S9) and mass for nitro- and oxy-PAH fractions of the extractable organic matter are presented in Table 5. Among samples studied, it is possible to observe that mutagenicity and mass of the fractions are not related themselves. Samples 2 and 4 presented the lowest mass for both nitro- and oxy-PAH fractions. Nevertheless, they had the highest mutagenic response. Low mass and high mutagenic activity found in most of the nitro-PAH fractions suggest the presence of highly mutagenic compounds in these fractions.

CONCLUSIONS

The oxy-PAH fractions, which contain more polar compounds than the nitro-PAH fractions, should have compounds containing oxy and/or hydroxyl radicals that could be even more mutagenic than the nitro-PAH compounds. Because such compounds were not detected by the chemical method used in this work, it is difficult to suggest which oxygenated mutagenic compounds are present in these samples collected in the atmosphere of São Paulo City. The response obtained in the mutagenicity assay indicated that there are compounds containing NO$_2$ radical in the molecules but certainly further investigation is still necessary for a better understanding of the prevalent mutagens in the oxy-PAH fractions.

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ABSTRACT

Occurrence of polycyclic aromatic hydrocarbons derivatives and mutagenicity study in extracts of PM$_{10}$ collected in São Paulo, Brazil

Nitro-PAH and oxy-PAH fractions in organic extracts of PM$_{10}$ collected in São Paulo City, Brazil (winter, 2004) were chemically and toxicologically analyzed. Some nitro-PAH compounds that are emitted from vehicular motor exhaust were quantified. The 3-nitrofluoranthene, which

![Figure 3.](image-url)

Figure 3. The relative contribution of the 1-NPy to the mutagenicity of the nitro-PAH fractions using *Salmonella typhimurium*, YG1041 strain, without S9 (a) and with S9 (b).

Table 5. Mutagenicity for YG1041 - S9 and mass of nitro- and oxy- PAH fractions.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Nitro-PAH fraction</th>
<th>Oxy-PAH fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mutagenicity (rev µ⁻¹)</td>
<td>Mass (µg)</td>
</tr>
<tr>
<td>1</td>
<td>99</td>
<td>3.09</td>
</tr>
<tr>
<td>2</td>
<td>880</td>
<td>0.24</td>
</tr>
<tr>
<td>4</td>
<td>1200</td>
<td>0.81</td>
</tr>
<tr>
<td>8</td>
<td>180</td>
<td>0.85</td>
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<td>9</td>
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</tbody>
</table>
probably coeluted with the 2-nitrofluoranthene, a secondary pollutant, presented higher levels (average: 0.16 ng m⁻³; maximum concentration: 0.26 ng m⁻³) than 1-nitropyrene (average: 0.031 ng m⁻³; maximum concentration: 0.054 ng m⁻³). No detectable amount of 6-nitrocrysene was found. Oxy-PAH compounds were not detected probably because they were present at concentrations much low. Both nitro- and oxy-PAH fractions have showed a similar frameshift mutagenic response with a higher potency in the absence of S9 compared to that in the presence of S9, indicating the prevalence of direct-acting compounds for the Salmonella/microsome assay. The responses observed for the YG1041 strain (up to 1200 revertants μg⁻¹) were significantly higher than for the TA98 (up to 28 revertants μg⁻¹) indicating the presence of nitroaromatic compounds. At last, the presence of highly mutagenic compounds in the nitro-PAH fractions and oxygenated compounds containing NO₂ radical in the oxy-PAH fraction was evidenced.

Keywords: Urban air pollution, particulate matter, nitro-PAH and oxy-PAH compounds, mutagenicity

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