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UHPLC-(+)APCI-MS/MS determination of oxygenated and nitrated polycyclic aromatic hydrocarbons in airborne particulate matter and tree barks collected in Buenos Aires city

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ABSTRACT

A study was undertaken to evaluate the content of oxygenated and nitrated polycyclic aromatic hydrocarbons (OPAHs and NPAHs) in airborne particulate matter (PM_{2.5} and PM₁₀), and tree barks. For PM_{2.5} and PM₁₀ collection different approaches were followed using different type of filters and sample collectors. Samples of PM_{2.5} were collected on glass fiber filters during one month with a medium volume sampler. For PM₁₀, glass and quartz fiber filters were used and samples were collected simultaneously for one week using a high volume sampler. In addition, bark samples were collected at the same sites. Ultra high performance liquid chromatography-atmospheric pressure chemical ionization tandem mass spectrometry (UHPLC-APCI-MS/MS) was used for PAHs derivatives quantification. Data acquisition under MS/MS was achieved by applying multiple reaction monitoring to provide a high degree of sensitivity and specificity. Chromatographic separation was performed on a reverse phase rapid resolution column using a gradient mode (total run time: 5.0 min). The study evidenced that in airborne particles matter, four PAHs derivatives were detected at concentrations from 0.01 to 240.62 ng m⁻³ (or 0.3 µg g⁻¹ to 30 mg g⁻¹). Meanwhile, in tree barks, only OPAHs were detected at concentrations varying from 0.18 to 0.72 µg g⁻¹.

Keywords: OPAHs; NPAHs; UHPLC-(+)APCI-MS/MS; APM; Tree Barks.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous semi-volatile organic compounds, partitioned in atmospheric aerosols between the vapor and particulate phase. They are formed in all incomplete combustion processes at temperatures > 500 °C with insufficient oxygen and are originated by natural (forest fires, volcanic activity) [1] and anthropogenic sources (fossil fuel combustion, industrial and agricultural activities, power generation, municipal and medical incineration) [2-4]. The widespread distribution and semi-volatile nature of PAHs, is connected to the fact that previous emissions that have deposited on various media (e.g. soil and vegetation) may be re-emitted into the atmosphere [1].

In urban areas the major contribution of PAHs, including their derivatives: oxygenated and nitrated polycyclic aromatic hydrocarbons (NPAHs and OPAHs), is vehicular traffic through incomplete combustion of fossil fuels namely, diesel and gasoline [5-7]. NPAHs can be either directly emitted from combustion (mainly diesel engines) or formed in the atmosphere via electrophilic nitration reactions of a PAH (in gas phase) in the presence of NO_2 , OH or NO_3 radicals [8]. NPAHs have been detected in airborne particles, coal fly ash, diesel engine exhaust emissions, kerosene heaters, and cigarette smoke. Once release into the atmosphere, NPAHs are highly persistent in the environment and can be transported long distances from their original source.

Oxygenated-PAHs can also be emitted directly by incomplete combustion or generated as the result of atmospheric reactions following oxidation of the corresponding PAHs or their derivatives with hydroxyl radicals and/or ozone. These derivatives are generally less volatile than their precursors (with a higher molecular weight and lower vapor pressures), and have a tendency to be associated to particles [9-11]. Recently, increasing interest has been paid to the study of OPAHs, particularly

quinones, because these derivatives are reported as being more toxic than their parent PAHs [10, 12, 13]. Because of this and also their persistence in the atmosphere, OPAHs have become key components in the study of airborne particulate matter (APM).

Nitrated and oxygenated PAHs are environmental pollutants of particular concern because of the recognized mutagenic, carcinogenic, and immunotoxic effects of a number of the individual compounds (termed congeners). Detrimental effects on human health have been reported [14]. For this reason, regulation of PAHs and their derivatives' emissions as well as reliable monitoring of these compounds in urban air is of prime importance from the human health standpoint. NPAHs have been reported as a class of very potent mutagenic compounds [15]. In a study carried out in the North-Eastern United States, OPAHs have been reported as the most mutagenic compounds regarding human-cell mutagens in respirable airborne particles [16].

Other alternative for OPAHs and NPAHs analysis in atmospheric particles is the use of biomonitors, which represent a simpler and cheaper choice in comparison with the high costs associated with conventional monitoring based on the use of high/medium/low volume samplers and expertise necessary for a proper use of these samplers. Several authors have been used tree barks, leaves, pine needles, lichens, and mosses as bioaccumulators or/and biomonitors to evaluate the PAHs and/or their derivatives concentrations in the environment [17-22].

During more than ten years, our group has conducted a series of studies aimed at determination, fractionation, and source identification of metals, metalloids, and ions in airborne particulate matter and related environmental matrices such as tree barks, road dust, and ashes [23-31]. In the framework of a new project, our interest is now focused on the contribution with information on the content of OPAHs and NPAHs in the

atmosphere of the megacity of Buenos Aires as well as the identification of their potential sources. To the best of our knowledge this is the first time that the presence of these compounds in atmospheric aerosols and tree barks is reported for the megacity of Buenos Aires. Moreover, some of the compounds studied have not been reported in the herein selected matrices elsewhere. Additionally, from an analytical point of view, the proposed work is completely novel in terms of comparing different extraction strategies, samples, and sampler devices; offering thus a sensitive, rapid, economical, and reliable tool for environmental monitoring of nitrated and oxygenated PAHs.

2. Experimental

2.1. Instrumentation

Mass spectrometry determinations were performed on a Quattro PremierTM XE Micromass MS Technologies tandem triple quadrupole mass spectrometer with an atmospheric pressure chemical ionization source (Waters, Milford, USA).

An AcquityTM Ultra High Performance LC system (Waters, Milford) equipped with an autosampler injection and pump systems (Waters, Milford, USA) was used. The autosampler vial tray was maintained at 4 °C. The separation was performed using an ACQUITY UPLC[®] BEH C18 (Waters, Milford, USA) analytical column (2.1 mm internal diameter × 50 mm length, and 1.7 µm particle size min⁻¹).

A Milestone Start D microwave system (Sorisole, Italy) was used for sample treatment. To accelerate the extraction of the PAHs, an ultrasonic bath (Cleanson 1106, Buenos Aires, Argentina) was employed.

2.2. Reagents

Chemical standards of environmentally relevant PAHs derivatives were selected. Thus 1-nitropyrene (1-NPYR), 2-nitrofluorene (2-NFLU), 3-nitrofluoranthene (3-NFLUANTH), 9-nitroanthracene (9-NANTH), 1,5-dinitronaphthalene (1,5-DNNAPHT), 2-fluorencarboxaldehyde (2-FLUCHO), 5,12-naphthacenequinone (5,12-NAPHTONA) and 9,10-anthracenequinone (9,10-ANTHRONA) were purchased from Sigma Chemical (St. Louis, MO, USA). The PAHs derivatives used in this study are shown in **Table 1**.

Acetonitrile, methanol, dichloromethane, toluene, chloroform, acetone, and water Optima[®] LC-MS grade were purchased from Fisher Scientific (Fair Lawn, New Jersey). Formic acid was obtained from Fisher Scientific (Loughborough, UK). Organic (acetonitrile-based) working standard solutions were prepared by stepwise dilution from 10.0 mg L⁻¹ stock standard solutions of each compound. Quantification was achieved by preparing spiked samples with proper amounts of the analytes.

2.3. Sample collection

2.3.1. Airborne particulate matter collection

Airborne particulate matter was collected during a sampling campaign carried out between July (winter in the Southern Hemisphere) and October (spring in the Southern Hemisphere) 2012. Fine-respirable (PM_{2.5}) and coarse-inhalable (PM₁₀) size ranges were collected. Samples were collected at one sampling site on the roof of a building at Comisión Nacional de Energía Atómica (CNEA) at an elevation of ~ 12 m. The selected site is located at ~ 15 m distance from a highway (34° 38'S, 58° 28'W). This site is placed at approximately 200 km from the open sea and influenced mainly by emissions from vehicular traffic and residential sources.

A total of 14 samples of PM₁₀ were collected during seven days in parallel using two (A and B) high-volume air samplers (SIBATA, HV 1000F, Japan) equipped with a PM₁₀ size-selective inlet. In sampler A, seven ash-free glass-fiber filters (8 x 10 inch, ADVANTEC) were used for particles collection. In sampler B, seven quartz fiber filters (8 x 10 inch, MILLIPORE) were employed. In both cases, the sampling flow rates used were 1000 L min⁻¹ and the collection period in each filter was 24 h (approximately from noon to noon of consecutive days) to achieve a concentration of PAHs derivatives adequate for their quantification. The average air volume daily collected was 1440 m³.

In addition, PM_{2.5} samples were collected during one month on glass-fiber filters using a medium-volume air sampler (PQ200, BGI Incorporated, MA USA) equipped with a PM_{2.5} size-selective inlet. The average air volume daily collected was 24 m³. Before collection each filter was placed in a stove at 400 °C by 5 h to eliminate any impurity. Then, all filters were placed under constant temperature (~ 25 °C) and relative humidity (□ 40%) for 24 h before weighing. After sampling, all filters were wrapped in aluminium foil and stored in a dry and dark place at 4 °C. Then, they were rapidly transported to the laboratory where were frozen until analysis.

2.3.2. Tree barks collection

Using a hard steel knife, the external surface of the bark of five *Tipa sp.* trees, not exceeding 2 mm in depth, was removed from the face mostly exposed to traffic, at a ~ 1-1.5 m height. Barks were collected using rubber gloves. After collection, the tree bark samples were wrapped in aluminium foil and placed in a dry and dark place at 4 °C. Then, the tree barks were rapidly transported to the laboratory where they were frozen until analysis.

2.4. Sample treatment

In a first step, to select the optimal technique for OPAHs and NPAHs extraction from filters and tree barks samples, two approaches were used: (i) microwave-assisted extraction: using hermetically sealed 1-cm wall thickness polytetrafluoroethylene reactors (100 mL internal volume), and (ii) ultrasonic-assisted-extraction. Also to determinate the optimal solvent or solvent mixtures to use, different combinations were assayed, evaluated, and compared: methanol, dichloromethane, methanol:acetone (1:1), methanol:toluene (4:6), methanol:toluene:acetone (1:1:1), and methanol:chloroform (1:1).

The microwave-based extraction approach was performed as follows: proper amounts of sample were placed in individual microwave reactors. Aliquots of 10 mL of the above mentioned solvents were added to the different vessels. Extraction was carried out at a ramp temperature up to 100 °C for 10 min and held for 30 more min. The employed microwave power was up to 1000 W. For the second approach, aliquots of 10 mL of solvent were added to each sample. Ultrasonication was employed to assist and accelerate the extraction of the compounds from filters and tree barks in an ultrasonic bath at 40 °C; during three cycles of 30 min each.

In both procedures, an aliquot of 2 mL of the resulting extract was dried under a N₂ stream, then 500 µL of acetonitrile with 0.1% (v/v) formic acid were added and the tubes were vortexed for 30 s. Finally, the reconstituted sample was filtered through 0.22 mm syringe filters (WatersTM) and stored in amber vials suitable for subsequent UHPLC-MS/MS analysis.

2.5. Polycyclic aromatic hydrocarbons derivatives analysis

2.5.1. Mass spectrometric determination

Determinations were performed on a tandem triple quadrupole mass spectrometer with an atmospheric pressure chemical ionization (APCI) source. The source was operated in a positive mode at 400 °C with N₂ as the nebulizer and the source temperature was kept at 120 °C. The corona discharge current was maintained at 3.0 µA and the extractor voltage was set at 4.0 kV. Ultrapure nitrogen was used as desolvation gas at a flow of 200 L h⁻¹. Argon was used as collision gas at a flow of 0.20 mL min⁻¹. General conditions are summarized in **Table 2**. Detection was performed in multiple reaction monitoring (MRM) mode of selected ions at the first (Q₁) and third quadrupole (Q₃). To choose the fragmentation patterns of m/z (Q₁) → m/z (Q₃) for the analytes in MRM mode, direct infusion (via syringe pump) into the MS of the analytes' standard solutions in acetonitrile was performed and the product ion scan mass spectra were recorded. The data were acquired using MassLynx Mass Spectrometry Software (Waters, Milford, USA).

2.5.2. Chromatographic separation

Due to the non-polar nature of the analytes, the binary mobile phases consisted of methanol with 0.1% (v/v) of formic acid (A) and acetonitrile with 0.1% (v v⁻¹) of formic acid (B) delivered at 0.15 mL min⁻¹. The C₁₈ gradient was started at an initial composition of 70% A and 30% B, then 2.2 min linear gradient to 30% A, held for 2.2 min. A return to the initial conditions was accomplished by a 0.2 min gradient to 70% A, where it was held for 0.4 min. Thus the four analytes detected were temporarily resolved within 3 min, being 5.0 min the total chromatographic run time (**Fig. 1**). The column was held at a temperature of 35 °C. Under these conditions, no sample contamination or sample-to-sample carryover was observed.

3. Results and Discussion

3.1. Comparison of sample preparation procedures

To carry out these experiments, samples were milled or cut, weighed (1 g of tree barks and 0.5 g of filters) and transferred to glass tubes. Two different sample pre-treatment approaches namely, MW-assisted extraction and ultrasonic-assisted extraction using the six solvents/mixtures described above were set up and compared to establish a reproducible, accurate, and rapid approach for the extraction and subsequent determination of the NPAHs and OPAHs under study in APM and tree barks.

The study evidenced that for extracting from filters both kinds of PAHs derivatives, the best alternative for quantitative recovery was ultrasonic-assisted-extraction using dichloromethane as extractant. While when the extraction of PAHs derivatives from tree barks was assessed, it was observed that the better choice for quantitative recovery was MW-assisted extraction with a solvent mixture of methanol:toluene (4:6). These procedures were adopted for subsequent samples treatment. By using these approaches, a quantitative recovery of the analytes was reached.

3.2. Selection of chromatographic conditions

Screening experiments showed that to obtain an efficient NPAHs and OPAHs derivatives chromatographic retention, a mixture of methanol/acetonitrile as gradient solvents was the best choice. In addition, this gradient was compatible with the necessary conditions for the APCI interface. To enhance the signal response, formic acid as mobile phase modifier was also employed. The use of 0.1% (v v⁻¹) formic acid led to obtain improved peak shapes and retention times. The use of a buffer solution improved retention time, peak shape, and sensitivity in comparison with the results

obtained without buffer. In conclusion, a 0.1% (v/v) formic acid concentration provided the maximum response for the generation of the protonated $[M]^+$ and $[M+H]^+$ (this latter only obtained for 2-FLUCHO) ions, which were used for further MRM experiments. The effect of the mobile phase flow rate on the separation/retention of the analytes was evaluated using van Deemter plots. Thus, a flow rate of 0.15 mL min^{-1} gave the best results in terms of chromatographic conditions and APCI efficiency. Under the optimal mentioned conditions, the analytes were eluted within 3.0 minutes from the column within a 5.0 minutes total run cycle, which was considerably shorter than the ones reported by Lintelmann et al. [32] and Mirivel et al. [33].

3.3. Optimization of MS parameters and MRM transitions

The best instrumental conditions that would allow identification of the PAHs derivatives under study in environmental samples at trace levels were evaluated. Thus, PAHs derivatives standard solutions (10 mg L^{-1}) in acetonitrile were introduced into the MS system at a flow rate of $25\text{ }\mu\text{L min}^{-1}$ via a syringe pump.

Identification of the parent ion was performed in the full-scan mode by recording mass spectra from m/z 150 to 400 in positive mode. For quantitative mass spectrometric detection, various identification points are desirable to meet the identification performance criteria. In this study, the fact of monitoring one precursor ion and several daughter ions for each compound fulfills the mentioned criteria giving the necessary specificity to identify a substance correctly. To evaluate the transitions in the MRM mode, the precursor and the product ions were selected according to the analytes fragmentation patterns. The MRM conditions were further optimized for each analyte to obtain maximum sensitivity and are shown in **Table 3**. The most sensitive transitions for each compound were selected for quantification.

3.4. Matrix effects

As known, one downside effect of ambient ionization-mass spectrometric detection is that the ionization process is susceptible to matrix signal suppression or enhancement. As a consequence, the liquid chromatography - mass spectrometry response obtained from a standard can differ significantly from matrix samples. The origin and mechanism of matrix effects are not fully understood and there are many possible sources for ion suppression reported in the literature. Approaches adopted to minimize the ion suppression effects include: (i) increase selectivity in the extraction procedure for matrix clean-up, (ii) more chromatographic retention of analytes, (iii) optimize type of buffer and its concentration, (iv) use flow-splitting or nanospray, (v) post-column addition, and (vi) use two-dimensional chromatography [34]. In this study, the effect of sample matrix was assessed by comparing the signal of the analytes in spiked samples with respect to the signal in pure solvent (acetonitrile). Thus, calibration curves from spiked matrix and spiked pure solvent samples were created. The percentage of the quotient of the slopes (b) in the spiked and solvent samples was used as an indicator of the extent of the ion suppression or signal enhancement, which was calculated as: $100 - (b_{spiked} / b_{solvent} \times 100)$.

No signal enhancement was observed, but a response reduction of approximately 40% due to matrix interference was observed for filters and none effect for bark samples. As a consequence, quantifications of the selected analytes were carried out following the standard addition method.

3.5. Determination of NPAHs and OPAHs in airborne particulate matter and tree barks

The amount of PAHs derivatives in the atmosphere depends on many factors namely, temperature, vapor and atmospheric pressures, as well as the concentration and

properties of the material collected. Although eight compounds were initially studied, only two oxygenated PAHs namely, 5,12-NAPHTONA (C1) and 2-FLUCHO (C2), and two nitrated PAHs: 1-NPYR (C3), and 9-NANTH (C4) were detected in both sizes of the atmospheric particles analyzed; the rest of the compounds were not present or below the instrumental sub-ppb detection limits.

In the case of tree barks, only the oxygenated forms, C1 and C2, were detected. It is important to notice that, to the best of our knowledge, no literature references have been found with information about the two mentioned oxy-compounds in this matrix, which constitutes a relevant contribution and an important aspect correlated to environmental monitoring. The results discussed from now on will focus on the detected analytes.

In order to assess the most convenient sampler and filter type for PM_{2.5} and PM₁₀ collection and subsequent determination of PAHs derivatives, two types of samplers (high-volume and medium-volume) were employed. Quartz and glass fiber filters were used and compared. The study evidenced that for both type of filters and both particle sizes of APM, the compound 9-NANTH (C4), exhibited the lower concentrations between 0.01 to 1.47 ng m⁻³. Similar values have been reported by Albinet et al. (2006) [35]. This compound was reported as highly photolysable which could explain the low levels found. On the contrary, 2-FLUCHO (C2) was the most abundant exhibiting a wide range of concentrations ranging from 0.09 to 240 ng m⁻³. Oda et al. [36] and Sierra and coworkers [37] reported concentrations of ~1.6 ng m⁻³ for Kurashiki city (Japan) and ~0.6 ng m⁻³ for Santiago city (Chile). In general terms, the herein obtained values for 2-FLUCHO are higher in comparison with the already reported levels. Further studies will be carried out in order to find possible explanations

for these findings. Concentrations of 5,12-NAPHTONA (C1) and 1-NPYR (C3) were intermediate and similar to each other, in the range from 0.03 to 10.22 ng m⁻³.

According to Allen et al. (1997) [39], OPAH are generally distributed among aerosol size fractions based on their molecular weight. OPAH with molecular weights greater than 248 are associated primarily with the fine aerosol fraction. When concentrations of 5,12-NAPHTONA (MW=258.27) were compared in both fractions measured in this study, the higher levels were found in PM_{2.5}. Only for this compound, concentrations in PM_{2.5} resulted higher than those measured in PM₁₀. A more detailed analysis including the concentrations (ng m⁻³) of the studied compounds for the different filters and particle sizes is shown in **Fig. 2**.

The results obtained for 1-NPYR levels were similar to those reported in the literature for other cities of the world, like Marseilles [40], Paris [11], Ho Chi Minh [41], Copenhagen [15], Milan [42], Tokyo [43]; among others. It has been observed that 1-NPYR tends to predominate in diesel exhausts and this compound has also been identified in used oils from light-duty diesel engines. Analyzed samples were collected close to a highway with important circulation of heavy and light duty trucks that burn diesel oil. The secondary formation of 1-NPYR by heterogeneous reactivity processes of pyrene with atmospheric oxidants was recently reported [11]. Nevertheless, this secondary contribution to total 1-NPYR levels can be considered minor in comparison with its primary origin. On the other hand, 9-NANTH is a tracer of particles collected from vehicular exhausts from diesel engines where it is likely to be formed upon high temperature electrophilic nitration. In the atmosphere, this compound is also a product of electrophilic nitration.

The identification of marker compounds can contribute with information about the impact of different sources and they are used to indicate the prevalence of gasoline or diesel engines as well as the relative contribution of traffic, and other sources. When PM_{10} was collected, no significant dependence on the type of filter was observed, with a slight trend to obtain higher levels of the compounds when quartz fiber filters were employed. In conclusion and taking into account the above findings, the cost of the filters (much higher for quartz fiber filters), the mass collected (much lower when $PM_{2.5}$ is collected), and the concentrations measured (**Fig. 2**), we consider that for our future studies devoted to analyze PAHs derivatives, glass fiber filters in conjunction with a high volume sampler is the best alternative when PM_{10} is collected.

Temporal distribution of the compounds analyzed showed a homogeneous pattern since the concentrations of PAHs derivatives exhibited similar levels comparing the days of the week when samples were collected (**Fig. 3**).

Concentrations of the four compounds under study expressed in mass $mass^{-1}$ were as follows: 9-NANTH ($50 \mu g g^{-1}$ - $30 mg g^{-1}$) > 2-FLUCHO ($7.53 - 2646.9 \mu g g^{-1}$) > 5,12-NAPHTONA ($0.3 - 1368 \mu g g^{-1}$) > 1-NPYR ($0.2 - 442 \mu g g^{-1}$).

When tree barks were analyzed, only 5,12-NAPHTONA (C1) and 2-FLUCHO (C2) were detected at $\mu g g^{-1}$ concentration levels ranging from 0.23 to 0.72 for C1 and from 0.18 to 0.70 for C2. In addition, the concentrations (in $\mu g g^{-1}$) of PAHs derivatives detected in tree barks resulted considerably lower in comparison with those found in the mass collected on filters. Even when the employment of biomonitors is cheap and simple, much more information can be obtained when filters are used for sample collection. **Fig. 4** shows the concentrations (mass $mass^{-1}$) of C1 vs C2 measured in filters ($PM_{2.5}$ and PM_{10}) and tree barks where three groups can be identified. The

observed trend and a relatively high Pearson correlation coefficient (0.6) could indicate a possible common source of the studied compounds.

4. Conclusions

A rapid, sensitive and reliable method for the determination of oxygenated and nitrated polycyclic aromatic hydrocarbons in environmental matrices such as airborne particulate matter (PM_{2.5} and PM₁₀) and tree barks by UHPLC-(+)APCI-MS/MS was developed.

For extracting both kinds of PAHs derivatives from filters, the best alternative was ultrasonic-assisted-extraction using dichloromethane; while for tree barks, the best choice was MW-assisted extraction with a solvent mixture of methanol:toluene.

Eight compounds were initially evaluated, but only two oxygenated and two nitrated forms: 5,12-NAPHTONA, 2-FLUCHO, 1-NPYR, and 9-NANTH; respectively, were detected in both sizes of the atmospheric particles analyzed. In the case of tree barks, only the two oxygenated forms were detected. The study showed that even when biomonitoring is cheap and simple to handle, much more information is obtained when filters are used for sample collection.

PAHs derivatives concentrations resulted significantly lower in the fine fraction in comparison with those measured in the coarse fraction. This latter is an indicative that PAHs derivatives are present in both fractions. On the other hand, when concentrations were assessed, the obtained values for PM_{2.5} resulted higher than those those for PM₁₀, indicating a higher enrichment of PAH derivatives in the fine fraction.

This is an important point to consider because of the risks associated with fine particles from the health standpoint of view.

Taking into account the results of this study, the cost of the filters (much higher for quartz fiber filters), the mass collected (much lower for $PM_{2.5}$), and the concentrations measured; it is possible to conclude that glass fiber filters, in conjunction with a high volume sampler, is the best alternative when PM_{10} is collected. In addition, given the enrichment of PAHs derivatives in $PM_{2.5}$, it is of utmost importance to keep studying the levels of these compounds in this fraction.

Despite the restricted number of samples analyzed some conclusions on the contribution of traffic source indicators could be derived from the study. Concentration of the analyzed PAHs derivatives resulted to be higher than those reported for other cities indicating an exposure of the inhabitants of Buenos Aires city to these pollutants.

To the best of our knowledge this study reports for the first time the presence of 1-NPYR, 9-NANTH, 2-FLUCHO and 5,12-NAPHTONA in atmospheric particles ($PM_{2.5}$, PM_{10}) and tree barks collected in the city of Buenos Aires, which adds information on the presence of these compounds in urban atmospheres.

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References

- [1] A.I. Barrado, S. García, Y. Castrillejo, E. Barrado, Exploratory data analysis of PAH, nitro-PAH and hydroxy-PAH concentrations in atmospheric PM10-bound aerosol particles. Correlations with physical and chemical factors, *Atmospheric Environment* 67 (2013) 385-393.
- [2] K.F. Souza, L.R.F. Carvalho, A.G. Allen, A.A. Cardoso, Diurnal and nocturnal measurements of PAH, nitro-PAH, and oxy-PAH compounds in atmospheric particulate matter of a sugar cane burning region, *Atmospheric Environment* 83 (2014) 193-201.
- [3] H.L. Chiang, Y.M. Lai, S.Y. Chang, Pollutant constituents of exhaust emitted from light-duty diesel vehicles, *Atmospheric Environment* 47 (2012) 399-406
- [4] M.S. Alam, J.M. Delgado-Saborit, C. Stark, R.M. Harrison, Using atmospheric measurements of PAH and quinone compounds at roadside and urban background sites to assess sources and reactivity, *Atmospheric Environment* 77 (2013) 24-35.
- [5] S.R. Wild, K.C. Jones, Polynuclear aromatic hydrocarbons in the United kingdom environment: a preliminary Source inventory and budget, *Environmental Pollution* 88 (1995) 91-108.
- [6] World Health Organization 2004, Environmental Health Criteria 229, Selected nitro- and nitro-oxy- polycyclic aromatic hydrocarbons
http://www.who.int/ipcs/publications/ehc/ehc_229/en/
- [7] M.S. Nocun, M.M. Schantz, Determination of selected oxygenated polycyclic aromatic hydrocarbons (oxy-PAHs) in diesel and air particulate matter standard reference materials (SRMs), *Anal. Bioanal. Chem.* 405 (2013) 5583-5593.
- [8] K. Zimmermann, N. Jariyasopit, S.L. Massey Simonich, S. Tao, R. Atkinson, J. Arey, Formation of Nitro-PAHs from the Heterogeneous Reaction of Ambient

- Particle-Bound PAHs with N₂O₅/NO₃/NO₂, *Environ. Ciencia. Technol.* 47 (15) (2013) 8434-8442.
- [9] R. Atkinson, J. Arey, Gas-phase tropospheric chemistry of biogenic volatile organic compounds: a review, *Atmospheric Environment* 37 (2003) 197-219.
- [10] C. Walgraeve, K. Demeestere, J. Dewulf, R. Zimmermann, H.V. Langenhove, Oxygenated polycyclic aromatic hydrocarbons in atmospheric particulate matter: Molecular characterization and occurrence, *Atmospheric Environment* 44 (2010) 1831-1846.
- [11] J. Ringuet, E. Leoz-Garziandia, H. Budzinski, E. Villenave, A. Albinet, Particle size distribution of nitrated and oxygenated polycyclic aromatic hydrocarbons (NPAHs and OPAHs) on traffic and suburban sites of a European megacity: Paris (France), *Atmos. Chem. Phys.* 12 (2012) 8877-8887.
- [12] K.C. Tagliari de Brito, C. Torres de Lemos, J.A. Vaz Rocha, A.C. Mielli, C. Matzenbacher, V.M. Ferrão Vargas, Comparative genotoxicity of airborne particulate matter (PM_{2.5}) using *Salmonella*, plants and mammalian cells, *Ecotoxicology and Environmental Safety* 94 (2013) 14-20.
- [13] T.S. Pereira, L.S. Beltrami, J.A.V. Rocha, F.P. Broto, L.R. Comellas, D.M.F. Salvadori, V.M.F. Vargas, Toxicogenetic monitoring in urban cities exposed to different airborne contaminants, *Ecotoxicology and Environmental Safety* 90 (2013) 174-182.
- [14] X. Zhu, G. Pfister, B. Henkelmann, J. Kotalik, S. Bernhöft, S. Fiedler, K.W. Schramm, Simultaneous monitoring of profiles of polycyclic aromatic hydrocarbons in contaminated air with semipermeable membrane devices and spruce needles, *Environmental Pollution* 156 (2008) 461-466.

- [15] A. Feilberg, M.W.B. Poulsen, T. Nielsen, H. Skov, Occurrence and sources of particulate nitro-polycyclic aromatic hydrocarbons in ambient air in Denmark, *Atmospheric Environment* 35 (2001) 353-366.
- [16] D.U. Pedersen, J.L. Durant, B.W. Penman, C.L. Crespi, H.F. Hemond, A.L. Lafleur, G.R. Cass, Human-cell mutagens in respirable airborne particles in the Northeastern United States. 1. Mutagenicity of fractionated samples, *Environ. Sci. Technol.* 38 (2004) 682-689.
- [17] E. Lehndorff, L. Schwark, Biomonitoring airborne parent and alkylated three-ring PAHs in the Greater Cologne Conurbation II: Regional distribution patterns, *Environmental Pollution* 157 (2009) 1706-1713.
- [18] E. Ötvös, I.O. Kozák, J. Fekete, V.K. Sharma, Z. Tuba, Atmospheric deposition of polycyclic aromatic hydrocarbons (PAHs) in mosses (*Hypnum cupressiforme*) in Hungary, *Science of the Total Environment* 330 (2004) 89-99.
- [19] M. Blasco, C. Domeño, C. Nerín, Use of Lichens as Pollution Biomonitoring in Remote Areas: Comparison of PAHs Extracted from Lichens and Atmospheric Particles Sampled in and Around the Somport Tunnel (Pyrenees), *Environ. Sci. Technol.* 40 (2006) 6384-6391.
- [20] S. Orecchio, PAHs associated with the leaves of *Quercus ilex* L.: Extraction, GC–MS analysis, distribution and sources Assessment of air quality in the Palermo (Italy) area, *Atmospheric Environment* 41 (2007) 8669-8680.
- [21] N. Ratola, S. Lacorte, D. Barceló, A. Alves, Microwave-assisted extraction and ultrasonic extraction to determine polycyclic aromatic hydrocarbons in needles and bark of *Pinus pinaster* Ait. and *Pinus pinea* L. by GC–MS, *Talanta* 77 (2009) 1120-1128.

- [22] S. Augusto, C. Máguas, J. Matos, M.J. Pereira, C. Branquinho, Lichens as an integrating tool for monitoring PAH atmospheric deposition: A comparison with soil, air and pine needles, *Environmental Pollution* 158 (2010) 483-489.
- [23] P. Smichowski, D.R. Gomez, L.E. Dawidowski, M.F. Giné, A.C. Sanches Bellato, S. Reich, Monitoring trace metals in urban aerosols from Buenos Aires city. Determination by plasma-based techniques, *J. Environ. Monit.* 6 (2004) 286-294.
- [24] F. Fujiwara, M. Dos Santos, J. Marrero, G. Polla, D. Gómez, L. Dawidowski, P. Smichowski, Fractionation of eleven elements by chemical bonding from airborne particulate matter collected in an industrial city in Argentina, *J. Environmental Monitoring* 8 (2006) 913-922.
- [25] D. Gómez, M. Dos Santos, F. Fujiwara, G. Polla, J. Marrero, L. Dawidowski, P. Smichowski, Fractionation of metals and metalloids by chemical bonding from particles accumulated by electrostatic precipitation in an Argentine thermal power plant, *Microchemical Journal* 85 (2007) 276-284.
- [26] P. Smichowski, G. Polla, D. Gomez, A.J. Fernandez Espinosa, A. Calleja López, A three-step metal fractionation scheme for fly ashes collected in an Argentine thermal power plant, *Fuel* 87 (2008) 1249-1258.
- [27] F. Fujiwara, D. Gómez, L. Dawidowski, P. Perelman, A. Faggi, Metals associated with airborne particulate matter in road dust and tree bark collected in a megacity (Buenos Aires, Argentina), *Ecological Indicators* 11 (2011) 240-247.
- [28] A. Faggi, F. Fujiwara, C. Anido, P. Perelman, Use of tree bark for comparing environmental pollution in different sites from Buenos Aires and Montevideo, *Environmental Monitoring and Assessment* 178 (2010) 237-245.

- [29] F. Fujiwara, R. Rebagliati, J. Marrero, D. Gómez, P. Smichowski, Antimony as traffic-related element in size-fractionated road dust samples collected in Buenos Aires, *Microchemical Journal* 97 (2011) 62-67.
- [30] F. Fujiwara, R. Jiménez Rebagliati, L. Dawidowski, D. Gómez, G. Polla, V. Pereyra, P. Smichowski, Spatial and chemical patterns of size fractionated road dust collected in a megacity, *Atmospheric Environment* 45 (2011) 1497-1505.
- [31] A. Londonio, F. Fujiwara, R. Jiménez Rebagliati, D. Gómez, P. Smichowski, Determination of mercury in size fractionated road dust samples by flow injection-cold vapour-atomic absorption spectrometry, *Microchemical Journal* 105 (2012) 77-82.
- [32] J. Lintelmann, K. Fischer, E. Karg, A. Schröppel, Determination of selected polycyclic aromatic hydrocarbons and oxygenated polycyclic aromatic hydrocarbons in aerosol samples by high-performance liquid chromatography and liquid chromatography-tandem mass spectrometry 381 (2) (2005) 508-519.
- [33] G. Mirivel, V. Riffault, J.C. Galloo, Simultaneous determination by ultra-performance liquid chromatography-atmospheric pressure chemical ionization time-of-flight mass spectrometry of nitrated and oxygenated PAHs found in air and soot particles, *Analytical and Bioanalytical Chemistry* 397 (2010) 243-256.
- [34] H. Trufelli, P. Palma, G. Famiglini, A. Cappiello, An overview of matrix effects in liquid chromatography-mass spectrometry, *Mass Spectrometry Reviews* 30 (3) (2011) 491-509.
- [35] A. Albinet, E. Leoz-Garziandia, H. Budzinski, E. Villenave, Simultaneous analysis of oxygenated and nitrated polycyclic aromatic hydrocarbons on standard reference material 1649a (urban dust) and on natural ambient air samples by gas

- chromatography–mass spectrometry with negative ion chemical ionisation, *Journal of Chromatography A* 1121 (2006) 106-113.
- [36] J. Oda, S. Nomura, A. Yasuhara, T. Shibamoto, Mobile sources of atmospheric polycyclic aromatic hydrocarbons in a roadway tunnel, *Atmospheric Environment* 35 (2001) 4819-4827.
- [37] M.D.R. Sienra M., Oxygenated polycyclic aromatic hydrocarbons in urban air particulate matter, *Atmospheric Environment* 40 (2006) 2374-2384.
- [38] S. Martin, L. Dawidowski, P. Mandalunis, F. Cereceda-Balic, D.R. Tasat, Characterization and biological effect of Buenos Aires urban air particles on mice lungs, *Environmental Research* 105 (2007) 340-349.
- [39] J. Allen, N. Dookera, K. Taghizadeh, A. Lafleur, K. Smith, A. Sarofim, Measurement of Oxygenated Polycyclic Aromatic Hydrocarbons Associated with a Size-Segregated Urban Aerosol, *Environmental Science & Technology* 31 (7) (1997) 2064-2070.
- [40] A. Albinet, E. Leoz-Garziandia, H. Budzinski, E. Villenave, Polycyclic aromatic hydrocarbons (PAHs), nitrated PAHs and oxygenated PAHs in ambient air of the Marseilles area (South of France): Concentrations and sources, *Science of the Total Environment* 384 (2007) 280-292.
- [41] T.T. Hien, L.T. Thanh, T. Kameda, N. Takenaka, H. Bandow, Nitro-polycyclic aromatic hydrocarbons and polycyclic aromatic hydrocarbons in particulate matter in an urban area of a tropical region: Ho Chi Minh City, Vietnam, *Atmospheric Environment* 41 (2007) 7715-7725.
- [42] P. Ciccioli, A. Cecinato, E. Brancaleoni, M. Frattoni, P. Zacchei, Formation and transport of 2-nitrofluoranthene and 2-nitropyrene of photochemical origin in the troposphere, *Journal of Geophysical Research* 101 (1996) 19567-19581.

- [43] H. Kakimoto, M. Kitamura, Y. Matsumoto, S. Sakai, F. Kanoh, T. Murahashi, K. Akutsu, R. Kizu, K. Hayakawa, Comparison of atmospheric polycyclic aromatic hydrocarbons and nitropolycyclic aromatic hydrocarbons in Kanazawa, Sapporo and Tokyo, *Journal of Health Science* 46 (2000) 5-15.

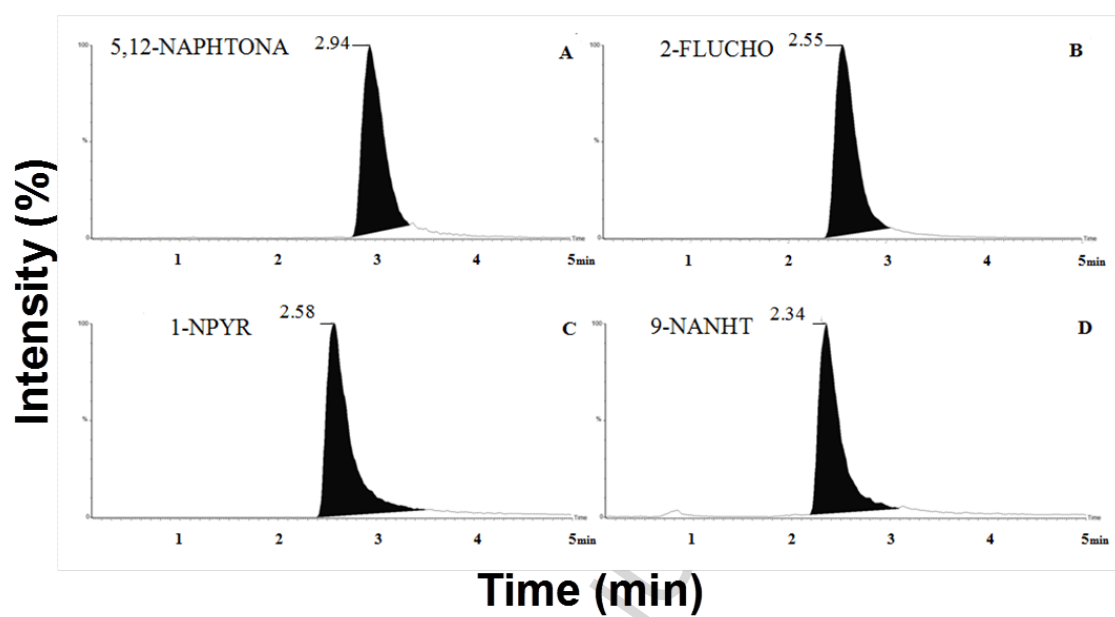


Fig. 1

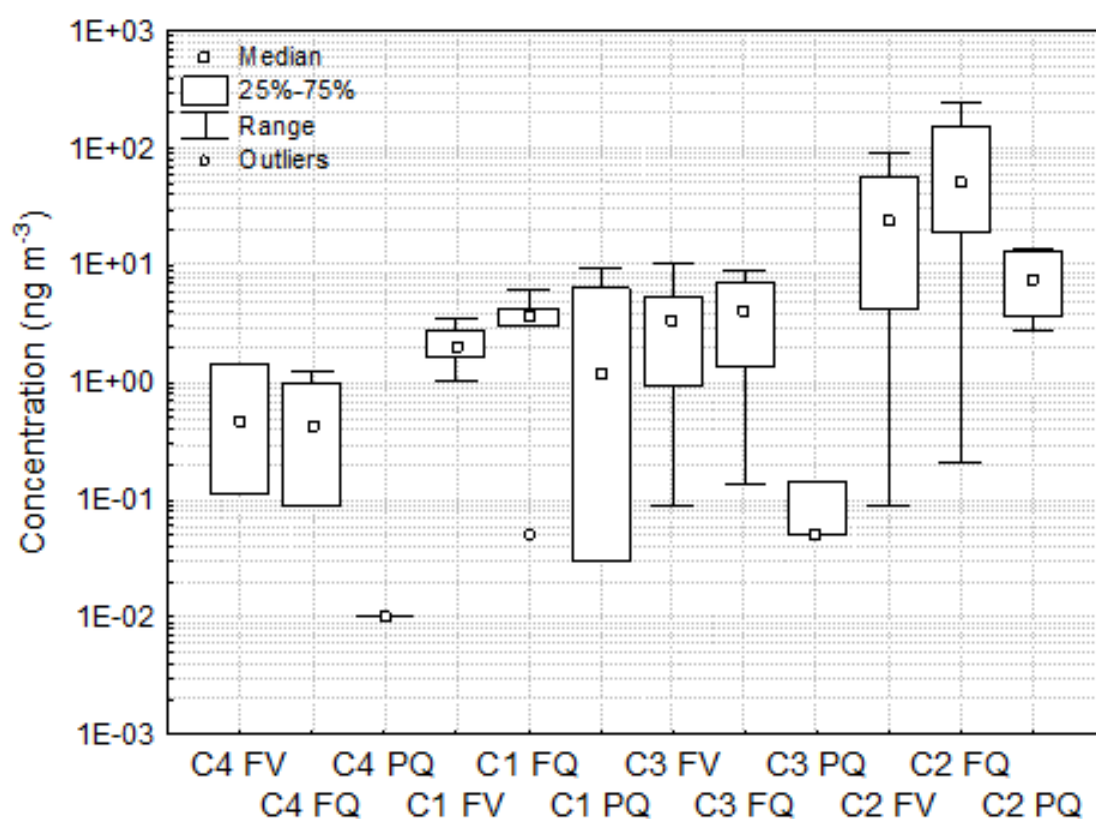


Fig. 2

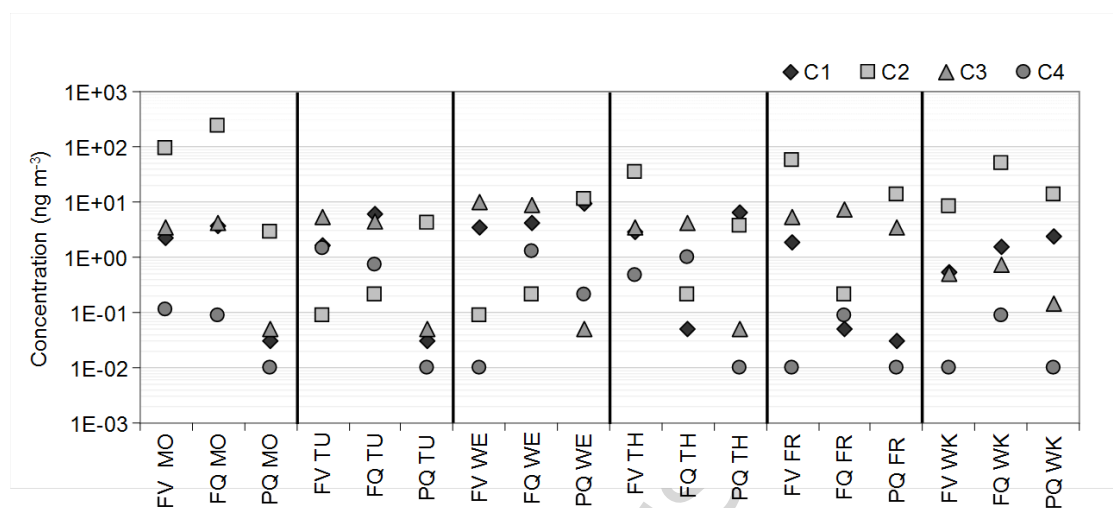


Fig. 3

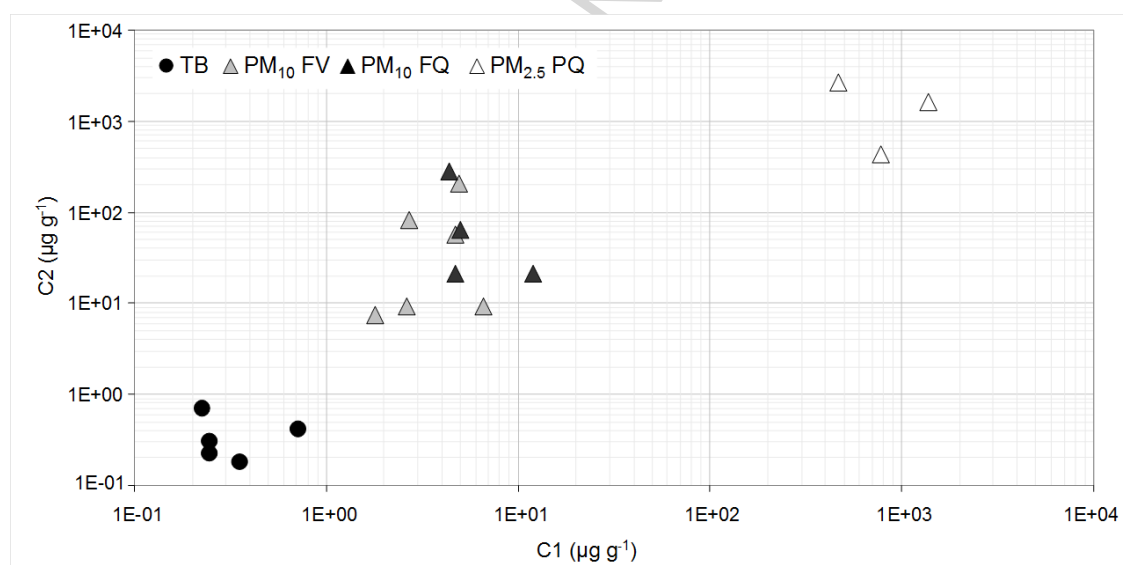


Fig. 4

Fig. 1. Chromatograms of the four compounds determined by UHPLC-(+)APCI-MS/MS: **a)** 5,12-NAPHTONA; **b)** 2-FLUCHO; **c)** 1-NPYR y **d)** 9-NANTH

Fig. 2. Concentration (ng m^{-3}) of the four compounds measured in PM_{10} using glass (FV) and quartz (FQ) fiber filters, and in $\text{PM}_{2.5}$ using glass fiber filters (PQ). C1: 5,12-NAPHTONA; C2: 2-FLUCHO; C3: 1-NPYR and C4: 9-NANTH.

Fig. 3. Weekly distribution of the four compounds collected in PM_{10} using glass (FV) and quartz (FQ) fiber filters, and in $\text{PM}_{2.5}$ using glass fiber filters (PQ). C1: 5,12-NAPHTONA; C2: 2-FLUCHO; C3: 1-NPYR and C4: 9-NANTH.

Fig. 4. Concentration ($\mu\text{g g}^{-1}$) of 2-FLUCHO vs. 5,12-NAPHTONA in tree barks (TB), PM_{10} collected in glass (FV) and quartz (FQ) fiber filters, and $\text{PM}_{2.5}$ collected in glass fiber filters (PQ).

Table 1. PAHs derivatives studied in this work

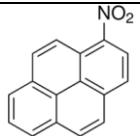

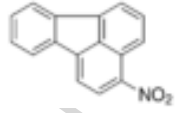
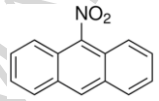
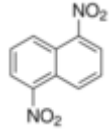
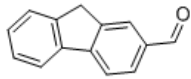
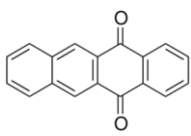
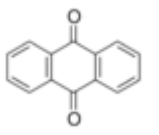
Compound	Empirical Formula	Structure	Abbreviation	Molecular weight (g mol ⁻¹)
1-nitropyrene	C ₁₆ H ₉ NO ₂		1-NPYR	247.25
2-nitrofluorene	C ₁₃ H ₉ NO ₂		2-NFLU	211.22
3-nitrofluoranthene	C ₁₆ H ₉ NO ₂		3-NFLUANTH	247.25
9-nitroanthracene	C ₁₄ H ₉ NO ₂		9-NANTH	223.23
1,5-dinitronaphthalene	C ₁₀ H ₆ (NO ₂) ₂		1,5-DNNAPHT	218.17
2-fluorencarboxaldehyde	C ₁₄ H ₁₀ O		2-FLUCHO	194.23
5,12-naphthacenequinone	C ₁₈ H ₁₀ O ₂		5,12-NAPHTONA	258.27
9,10-anthracenequinone	C ₁₄ H ₈ O ₂		9,10-ANTHRONA	208.21

Table 2. General (+)APCI-MS/MS experimental conditions for mass spectrometric determination of PAHs derivatives

Source temperatura (°C)	120
Corona current (μA)	3.0
Extractor voltage (V)	4.0
Desolvation temperatura (°C)	400
Desolvation gas flow [N ₂ , L h ⁻¹]	200
Collision gas flow [Ar, mL min ⁻¹]	0.20

Table 3. MRM (+)APCI-MS/MS experimental conditions for the detected compounds

Compound	Cone (V)	Precursor Ion (m/z)	Collision (V)	Product Ion (m/z)
5,12-NAPHTONA	30	[M] ⁺ =258	20	[M-2CO] ⁺ = 202 [*]
			20	[M-CO] ⁺ = 230
2-FLUCHO	27	[M+H] ⁺ = 195	15	[M+H-CO] ⁺ = 167 [*]
			30	[M-NO-CO] ⁺ = 189
1-NPYR	19	[M] ⁺ =247	50	[M-NO ₂] ⁺ = 201
			16	[M-NO] ⁺ = 217 [*]
9-NANTH	19	[M] ⁺ =223	16	[M-NO-CO] ⁺ = 165
			40	[M-NO ₂] ⁺ = 177
			16	[M-NO] ⁺ = 193 [*]

*Quantification transition

Highlights:

- Rapid and sensitive tool for the environmental monitoring of NPAHs and OPAHs.
- Evaluation of several sampling and analytes' extraction strategies.
- Novel compounds detected in the studied environmental samples.
- Experimental and ecotoxicological correlations and comparisons.