Lichens as biomonitors for the determination of polycyclic aromatic hydrocarbons (PAHs) in Caracas Valley, Venezuela

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Biomonitoring of PAH air pollution using lichens was carried out. Sixteen PAHs were studied in 11 locations along the valley of Caracas (Venezuela). The results of this work indicate that 14 of the 16 analysed PAHs were highly accumulated into the lichen thalli of *Pyxine coralligera* Malme. PAH levels in the samples revealed that the several volatile PAHs (naphthalene, acenaphtylene, acenaphtene, and fluoranthene) have the highest levels in the majority of the studied locations. The fluoranthene/pyrene and phenantrene/antracene ratios suggested that the major sources of PAHs are anthropogenic, mainly associated with gasoline and diesel combustion (pyrolytic) and unburnt oil derivates (petrogenic). The total PAH concentrations obtained in the present study were in the range of 0.24 to 9.08 mg/g, similar to those reported by other works in European and Asian cities.

Keywords: PAHs; atmospheric pollution; *Pyxine coralligera* Malme; fossil fuels; Caracas

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are organic compounds with two or more aromatic fused rings, mainly produced by incomplete pyrolysis of organic material from natural sources (e.g., wood combustion) [1] and anthropogenic emissions, by oil refining and by incomplete combustion of fossil fuels such as coal, petroleum hydrocarbon liquids or natural gas [2–5]. These compounds have been reported as ubiquitous atmospheric pollutants [6–7].

The International Agency for Research on Cancer has identified 16 polycyclic aromatic compounds with carcinogenic, teratogenic and mutagenic properties [8–9]. The studies of the behaviour of this kind of molecules in the environment are of primary importance to understand their effect on human health [10].

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Most of the studies on air pollutants have been carried out using equipments such as high-volume samplers; however such works bring out high costs and time consumption in installation in buildings and other locations, besides the difficulties of collecting samples in remote areas such as rural sites [11–12]. Moreover, data from these direct measurements do not necessarily provide a bioindicator response to pollutants. Much attention has been given to lichen species in biomonitoring surveys [13]. As indicated by several examples [14–17], the current international literature provides numerous works on the use of lichens as biomonitors of PAH air pollution.

In Venezuela, specially in the city of Caracas, pollutants such as metal and organic particulates have been studied using high-volume samplers for sample collection, but no work has been conducted with lichens as natural matrices for monitoring atmospheric deposition of this type of molecules [18–20].

The aim of this work has been to determine the possibility of using lichens as PAHs biaccumulators for the evaluation of atmospheric pollution in different areas of Caracas.

2. Experimental

2.1 Reagents

All the solvents (acetonitrile, hexane, cyclohexane, dichloromethane and pentane) were from Scharlab S.L. (Barcelona, Spain) and were of analytical purity and liquid chromatography grade. Silica gel (0.06–0.2 mm, 70–230 mesh) from Merck (Darmstadt, Germany) was activated for 16 h at 130 °C. The HPLC system was calibrated using an external standard method with a commercial solution of a standard mixture of the 16 US Environmental Protection Agency (EPA) priority PAHs (PAH Mix 9) in acetonitrile from Dr. Ehrenstorfer GmbH (Augsburg, Germany).

2.2 Lichen species

The selected lichen genus was *Pyxine* (Physciaceae, lichenised Ascomycota) due to its abundance in contaminated and non-contaminated sites in Caracas [21]. The main species of *Pyxine* in this city is *coralligera* Malme. Due to their small size and slow growth these lichens evolve naturally in places where higher plants have difficulties to grow. These species have been proven to accumulate elements such as Cr, Ni, Pb, Zn, Ca, Mn, Fe, Si, and Al as part of their structure. The reasons of their presence have been reported [22] due to the long-term exposition to geogenic sources and atmospheric conditions of a constant flow of liquids and gases. Furthermore, there is a physiological relationship between the environment and the lichen thalli, which is the reason that these species are able to concentrate and accumulate distinctive compounds [23].

2.3 Sampling and sample preparation

Tropical areas with high, medium, and low pollution and a control site (known as a very low pollution area) were selected for the study [24]. The location and description of the network are summarised in Table 1. An equal number of lichen samples were collected from 11 locations in Caracas (see Figure 1) during December 2007 and January 2008. A total of 33 samples were analysed. The main criteria used to select the sampling stations

Table 1. Location, sampling code, ambient conditions and average annual levels of PM_{10} (mg/m^3) corresponding to each sampling station.

Code	Location	Conditions	PM_{10}
lfr	La Floresta	High pollution	28
ljb	Botanical Garden	High pollution	26
lpc	Los Proćeres	High pollution	30
lps	Sucre Square, Catia	High pollution	28
lpm	Baralt Avenue	High pollution	27
lbv	Bolıv̌ar Avenue	Medium-high pollution	26
lpz	La Paz	Low-medium pollution	26
lsz	El Marques	Low-medium pollution	31
lcf	El Cafetal	Low-medium pollution	26
lpe	Prados del Este	Low polluttion	23
lusb	Simoń Bolıv̌ar Univ.	Very low pollution	21

Note: PM_{10} – breathable particles.

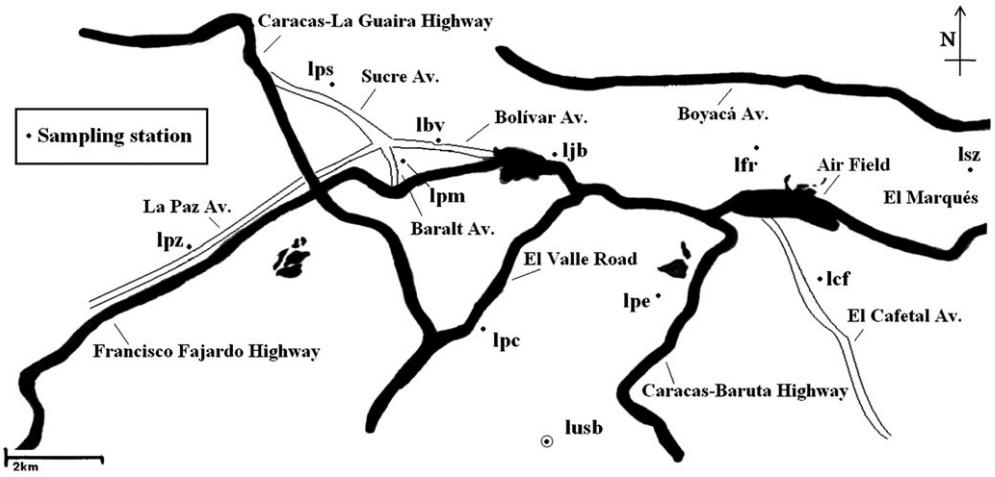

Figure 1. Caracas Valley, Venezuela.

were the different intensities of the vehicular traffic and also the air pollution by atmospheric particles (breathable particles, PM_{10}).

Twenty grams of natural lichens were collected from each of the above-mentioned areas and a portion (2 g) of each sample was used for subsequent homogenisation and analysis. Mature lichen samples and barks pieces were collected together from the tree trunks at 1 to 3.5 m from the ground; a standard stainless steel knife was used for this purpose. Then they were packaged and stored at about –20 °C in amber glass jars until their analysis [25]. In the laboratory the lichen samples were first cleaned by the use of a standard stereo zoom microscope in a Petri dish, removing the extraneous materials as for example leafage and pebbles. The samples were also transferred to 125 mL beakers and rinsed twice with 20 mL of Milli-Q water (15 min each time) with slow agitation, discarding the residual water. Finally, these samples were kept at 40 °C for 48 h in a stove [14]. Once they dried, they were crushed into an agate mortar for their homogenisation [26].

2.4 Sample extraction and purification

Two grams (± 0.0001 g) of each lichen sample (three replicates) were added to a 125 mL beaker and extracted with 30 mL of a cyclohexane-dichlorometane (4:1 v/v) mixture using an ultrasound bath (Cole-Parmer, Model 8851) at room temperature for 30 minutes. The resulting extract was filtered through a Whatman grade 40 quantitative filter paper. The solvent was slowly removed using a rotavapor at only 30 °C, avoiding operation under vacuum by using a nitrogen-gas stream until a volume of 2 mL [27]. No losses of the most volatile PAHs were confirmed after analysis of the condensed solvent in the evaporating flask of the rotary evaporator.

The final extract of 2 mL was purified by column adsorption chromatography (9 cm∼ 1 cm i.d.) using silica-gel (0.06–0.2 mm mesh) as stationary phase using a hexane slurry [28]. First, the aliphatic hydrocarbon fraction was eluted with 25 mL of n-hexane and it was discarded. Later, the polycyclic aromatic hydrocarbon fraction was eluted with 40 mL of a n-hexane-dichlorometane mixture (2:3 v/v). This second fraction was then reduced by rotary evaporator and nitrogen-gas flow until near dryness, and finally re-dissolved with 0.5 mL acetonitrile prior to chemical analysis by HPLC [29].

2.5 HPLC determination of PAH concentrations

The PAH concentrations were determined by High Performance Liquid Chromatography (HPLC) using a modular Waters chromatograph constituted by a 600s Controller and a 626 High Pressure Pump. A Waters 717-plus Autosampler, a specific PAH C18 S-5pm (250×4.6 mm) column, and both a FP 920 fluorescence detector and a 996 diode array detector, were also used. The gradient program consists of: first, acetonitrile-water (70:30) for 15 minutes, then an increase of the concentration of acetonitrile from 70 to 100%, maintaining these conditions for 13 minutes. The total elution time was of 30 minutes. The use of the more sensitive fluorescence detector in sequence with the diode array detector made possible the simultaneous analysis of the EPA priority PAHs. The non-fluorescent PAHs were quantified by using the diode-array equipment. The chromatographic peaks were identified at two absorption wavelengths, 254 nm and 208 nm, by using the diode array detector and at 375 and 425 nm as excitation and emission wavelength in fluorescence detector, respectively.

2.6 Method performance

Calibration of the analytical method was performed using external working solutions prepared from a commercial PAH-Mix 9 solution containing the 16 EPA priority polycyclic aromatic hydrocarbons prepared in acetonitrile/HPLC-grade. The limits of detection (LOD) were found in the range of 0.008 to 0.519 mg/g, whereas the limits of quantitation (LOQ) were between 0.25 and 1.73 mg/g, depending on the analysed PAH. The LOD and LOQ determinations were based on linear regression curves of measured data [30], considering the detection system used (diode array or fluorescence).

A recovery study was carried out on the three clean lichen samples collected from the control site (lusb). Two grams of each lichen sample were spiked adding 1 mL of the 5 mg/mL stock PAH solution until a final concentration of 2.5 mg/g. These lichen samples were spiked before extraction to simulate as much as possible the natural samples. The spiking procedure were performed in 125 mL beakers and kept at 25 °C about 16 hours in order to

Table 2. Mean recovery (%) and experimental data (mg/g) of each EPA PAH for the three spiked lusb samples prepared at 2.5 mg/g.

PAH	Mol. wt.	Exper. data	Stand. Dev.	Recovery*	Stand. Dev.
Naph	128.2	1.78	±0.01	71.0	±0.2
Ace	154.2	2.13	±0.11	85.4	±4.3
Acy	152.2	2.28	±0.04	91.3	±1.7
Fluo	166.2	1.95	±0.05	78.1	±1.9
Phen	178.2	2.13	±0.20	85.3	±8.0
Ant	178.2	1.63	±0.18	65.3	±7.3
Flt	202.3	2.28	±0.32	91.4	±12.8
Pyr	202.1	2.02	±0.17	81.0	±6.8
BaA	228.3	2.08	±0.30	83.2	±12.0
Chry	228.3	2.24	±0.24	89.5	±9.5
BbF	252.3	2.31	±0.34	92.2	±13.5
BkF	252.3	2.34	±0.34	93.7	±13.7
BaP	252.3	2.27	±0.34	90.7	±13.8
DahA	278.3	2.41	±0.41	96.3	±16.2
BghiP	276.4	2.34	±0.34	93.6	±13.4
IcdP	276.4	2.36	±0.34	94.4	±13.4

Note: *Range of AOAC (Association of Analytical Communities) from 65 to 115%.

allow solvent evaporation and bonding of PAHs into the biological lichen structure. The above-mentioned 5 mg/mL stock solution was initially added to each 2-gram lichen sample under slow agitation in order to mix them up as much as possible. Table 2 shows the average recovery and standard deviation of each polycyclic aromatic hydrocarbon classified according to three groups depending on their molecular weight: those of low molecular weight (166.2 g/mol or less), medium molecular weight polycyclic aromatic compounds (from 178.2 to 228.3 g/mol), and heavy PAHs (molecular weights over 252.3 g/mol).

2.7 Multivariate statistical analysis

Principal component analysis (PCA) was applied using the matrix formed by the 30 lichen samples not collected from the control site and the 14 EPA priority PAHs detected in them. The data matrix was standardised prior to PCA performance. Then, the principal components (PCs) were extracted according to various quality criteria: the number of eigenvalues higher than 1 (Kaiser criterion), the over 10–20 per cent of total variance and the over 65–75 per cent of accumulated variance explained by the corresponding PCs are given. PC extractions were carried out using a Varimax rotation. This multivariate analysis was performed using the CSS statistical software package provided by StatSoft®.

3. Results and discussion

3.1 PAH concentrations in lichens

Figure 2 shows a characteristic HPLC chromatogram of a real sample obtained by diode array detection. Table 3 shows the concentrations of 14 EPA priority PAHs identified and quantified in lichen samples which were collected from all sampling stations. As expected,

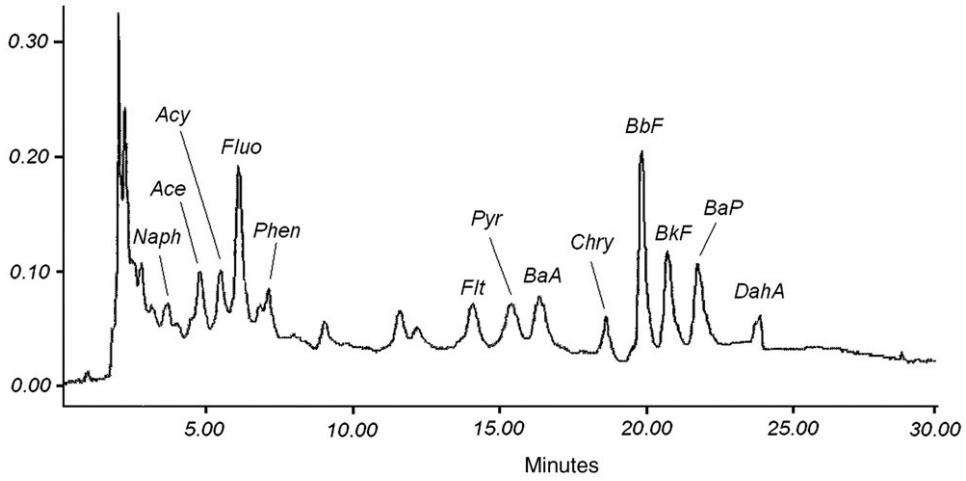

Figure 2. A characteristic HPLC chromatogram of a real sample obtained by diode array detection.

none of the above-mentioned PAHs were detected in samples from the control site (lusb). Benz(g,h,i)perylene and indeno(1,2,3-c,d)pyrene were not detectable in any samples.

Significant levels of several low molecular weight EPA priority PAHs were found in different sites: acenaphtene in lcf, lbv and lfr; naphtalene in lcf, lsz, lpm, lbv, lpz and lpc; acenaphthylene in lsz, lcf, lpz, lbv, lfr and lpc; and fluorene in lsz, lcf, lpz and lbv. These sampling sites are located along the east–west corridor of Caracas (see Figure 1), near heavily trafficked roads. Likewise, lcf and lsz locations display the highest values of light EPA priority PAHs and the highest total PAH data. In contrast, low levels of the mentioned PAHs were found in lps, ljb and mainly in lpe and lusb (control site). Most of these sites are located in the suburbs of Caracas (about 10–15 km from the city centre): lpe and lusb in the south end, lps in the northwest end. The distinctive PAH levels in the study locations could be partially explained by the different building and road patterns between the city centre and its surroundings. The only exception is the ljb site, which its low levels found may be due to the special location of this sampling site (inside the botanical garden) where numerous plant species can retain air pollutants. These results suggest preferential accumulation of low molecular weight PAHs by *Pyxine coralligera* Malme.

The medium molecular weight EPA priority PAHs showed the lowest concentrations in the lichen samples, though fluoranthene levels were moderate and high in lsz and lpm locations, respectively, whereas chrysene level was moderate in lpm. Phenantrene was detected in all sampling sites (mainly in lsz, lpc and lps) except in lusb. Anthracene displayed the lowest level in every location. In addition, very low levels of benz[a]antracene were also found in the majority of sampling sites. Phenantrene is specifically associated with diesel (pyrolytic) from vehicular emissions [32–33]. Migaszewski *et al.* [34] reported similar results using the lichen species *Hypogymnia physodes*, and discussed that the lichen thalli are efficient accumulators of medium molecular weight PAHs, mainly fluoranthene and phenanthrene; however, anthracene shows a lower efficiency. Similar results on PAHs of medium molecular weight have also been reported with the lichen species *Pseudevernia furfuracea* [14]. These authors concluded that those PAHs are mainly present in the gas phase due to their high vapour pressure, being easily accumulated by lichens.

Table 3. Concentrations (mg/g) of the PAHs detected in lichen samples collected in the 11 sampling sites.

					EPA priority PAH										
Station	Naph	Ace	Acy	Fluo	Phen	Ant	Flt	Pyr	BaA	Chry	BbF	BkF	BaP	DahA	Total PAHs
lps	0.68	n.d.	n.d.	n.d.	0.26	0.09	n.d.	0.22	0.23	n.d.	0.31	n.d.	n.d.	n.d.	1.79
lpe	n.d.	n.d.	n.d.	n.d.	0.12	0.12	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.24
lpz	1.09	n.d.	1.29	1.22	0.18	0.03	0.02	n.d.	0.03	0.04	0.27	n.d.	n.d.	n.d.	4.17
lsz	1.24	n.d.	1.48	1.48	0.31	0.01	0.44	n.d.	n.d.	n.d.	0.72	1.34	n.d.	n.d.	7.02
lbv	1.14	2.87	0.98	0.90	0.08	n.d.	n.d.	n.d.	0.28	n.d.	0.28	n.d.	n.d.	n.d.	6.53
lpm	1.15	n.d.	n.d.	0.34	0.14	0.03	1.11	n.d.	0.1	0.50	0.14	0.09	0.06	n.d.	3.66
lcf	1.90	3.06	1.39	1.31	0.12	0.01	n.d.	n.d.	n.d.	n.d.	0.45	0.84	n.d.	n.d.	9.08
ljb	0.10	0.60	0.34	0.37	0.05	n.d.	0.14	0.16	0.16	0.14	0.40	0.27	0.27	0.12	3.12
lpc	0.88	n.d.	0.72	0.69	0.32	0.09	n.d.	0.13	0.17	n.d.	0.95	0.97	1.82	n.d.	6.74
lfr	0.50	2.06	0.85	0.43	0.06	n.d.	n.d.	0.30	0.06	n.d.	0.16	0.17	n.d.	0.47	5.06
Average	0.96	2.15	1.01	0.84	0.16	0.05	0.43	0.20	0.15	0.23	0.41	0.61	0.72	0.30	4.74
Median	1.09	2.47	0.98	0.80	0.13	0.03	0.29	0.19	0.16	0.14	0.31	0.56	0.27	0.30	4.62
Range	0.10–1.90	0.60–3.06	0.34–1.48	0.34–1.48	0.05–0.32	0.01–0.12	0.02–1.11	0.13–0.30	0.03–0.28	0.04–0.50	0.14–0.95	0.09–1.34	0.10–1.90	0.60–3.06	0.24–9.08

Note: n.d. ¼ not detected.

Previously, Allen *et al.* [35] and Orlinski [36] had identified naphtalene, acenaphtylene, fluorene, phenanthrene, anthracene and fluoranthene in pine needles in urban atmospheres in different sites in Europe.

Several heavy EPA priority PAHs have been identified in the majority of the samples because a part of the atmospheric particulate matter deposited onto lichen surfaces – mainly fine particles containing usually these PAHs – could not have been removed by washing with Milli-Q water. High levels of benzo[b]fluoranthene and benzo[k]fluoranthene were found in lsz, lpc and lcf locations. Furthermore, benzo[a]pyrene was notably accumulated in lichen samples collected from lpc site. The accumulation of heavy PAHs in lichens may be positively related, in part, to their physicochemical properties such as the molecular weight and the octanol-water partition coefficient. Various authors [29] have concluded that the high molecular weight PAHs showed a special adsorption on surface of atmospheric particles, which were deposited later on the lichen surface. Likewise, Niu *et al.* [37] suggested that other climatic factors, such as precipitations, annual insolation or temperatures could be related to PAH dissolution, degradation or volatilisation. Consequently, high solar radiation and temperatures in Caracas valley could be affecting to the capture mechanism of semivolatile PAHs by lichens.

Recently, different authors [15–16] have reported values of total PAH concentration in the ranges of 0.70 to 6.24 mg/g and 3.38 to 25.01 mg/g in two sites located in Europe (Pyrenees) and Asia (Himalayas), respectively. It should be noted that values found in the atmosphere of Caracas city (0.24–9.08 mg/g) are similar to those found in both areas (Pyrenees or Himalayas). These relative low values in Caracas valley can be explained by the dynamics of the atmosphere, abundant precipitation and wind pattern. The high solar radiation intensity could lead to a high photochemical degradation of more labile PAHs, as it can be observed in the case of phenanthrene compared to anthracene [38].

3.2 Principal component analysis and PAH ratios

Table 4 shows the results of PCA. Three PCs were extracted explaining 87% of the total variance. The first and second PCs (PC1 and PC2) accounted for 44% and 22%, respectively, of the total variance and appeared grouping the light PAHs (highest score values were obtained for samples collected from lcf, lsz and lpz) and the medium molecular weight PAHs (lpm station), respectively. Finally, the last PC (PC3) explained 21% of total variance and grouped the heavy PAHs plus phenanthrene (lpc and lsz). Consequently, PCA tool confirms the results of the present work and leads to classify EPA priority PAHs according to their molecular weight.

The fluoranthene/pyrene and phenantrene/anthracene ratios have been used by different authors as an indicator of PAH sources [15]. A fluoranthene/pyrene ratio of 51 for ljb sampling site and phenantrene/antracene values of 510 for lps, lpe, lpz, lpm and lpc may indicate that pollution by PAHs is due to vehicular emissions and human (both industrial and domestic) activities with strong pyrolitic input (light and heavy PAHs can originate from the combustion of gasoline and diesel, respectively) [16]. Maertens *et al.* [39] found similar results. The average phenantrene/antracene ratio for the five above-mentioned stations was 3.6, which is consistent with nearby urban sites [40]. Finally, phenantrene/antracene ratios for lcf and lsz stations were characterised by values 410 which can be characteristic of petrogenic PAH pollution. These two sites are located near

Table 4. PCA of the PAH data obtained from sampling sites in Caracas.

Variables	PC1	PC2	PC3
Naph	0.85	0.24	0.10
Ace	0.74	—0.35	0.39
Acy	0.92	—0.31	0.09
Fluo	0.96	—0.08	0.16
Phen	0.14	0.04	0.81
Ant	0.57	0.53	—0.03
Flt	0.04	0.98	0.01
Pyr	0.43	0.68	—0.08
BaA	0.05	0.35	0.23
Chry	0.19	0.95	0.14
BbF	0.35	—0.12	0.90
BkF	0.39	—0.01	0.76
BaP	—0.21	—0.10	0.86
DahA	0.09	—0.50	0.56
Eigenvalue	4.0	2.0	1.9
Total variance (%)	44.4	21.8	20.9
Cum. variance (%)	44.4	66.2	87.1
Groups	Fluo, Acy, Naph, Ace, A t	Flt, Chry, Pyr	BbF, BaP, Phen, BkF, DahA
Observations	LMW þ Ant	MMW	HMW þ Phen
High-score cases	lcf, lsz, lpz	lpm	lpc, lsz

Notes: LMW ¼ low molecular weight; MMW ¼ medium mol. weight; HMW ¼ high mol. weight.

the eastern end of the Caracas valley where there are oil industry activities which probably constitute a significant source of pollution by PAHs.

4. Conclusions

It was of great interest for future investigations to evaluate if lichens may be used as natural traps that can retain PAHs in areas of Caracas valley. The present work shows the importance of *Pyxine coralligera* Malme lichens as bioaccumulators of air organic pollutants such as PAHs. This type of trap allows one to assess these compounds in the ambient air in a more efficient and economic way than other types. Results indicate that low molecular weight PAHs were those better accumulated in lichen samples. The highest values of the more volatile PAHs are probably due to their higher atmospheric gas phase concentrations.

According to the PAH concentrations and the statistical data, it can be concluded that different pollution levels are found in the sampling sites. In general, the highest PAH concentrations correspond to the highest traffic locations (by the city centre). The total PAH concentrations obtained in this study range from 0.24 to 9.08 mg/g. The values of fluoranthene/pyrene and phenantrene/anthracene ratios suggested that the major sources of PAHs are anthropogenic, petrogenic and mainly pyrogenic. It can also be established that low molecular weight compounds may come from gasoline combustion, whereas the heavy PAHs originated possibly from diesel engine emissions.

The results obtained in this study emphasise the utility and applicability of the *Pyxine coralligera* Malme lichens as bioindicators of air pollution by PAHs. It is also important to

claim the need of additional studies in order to evaluate the spatial and seasonal distribution of these pollutants in the whole Caracas valley and to identify additional sources that could be affecting this area.

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