Seasonal variations of PAHs content and distribution patterns in a mixed land use area: A case study in NE Italy with the transplanted lichen *Pseudevernia furfuracea*

Danijela Kodnik [a], Fabio Candotto Carniel [a, 1], Sabina Licen [b], Arianna Tolloi [b], Pierluigi Barbieri [b], Mauro Tretiach [a, *]

[a] *University of Trieste, Department of Life Sciences, Via L. Giorgieri 10, I-34127 Trieste, Italy*
[b] *University of Trieste, Department of Chemical and Pharmaceutical Sciences, Via L. Giorgieri 1, I-34127 Trieste, Italy*

HIGHLIGHTS

- Seasonal differences of the PAHs accumulation in lichen transplants were investigated.
- Important differences in concentration and distribution were observed.
- In summer PAH degradation processes are more pronounced and some sources are inactive.
- Environmental monitoring of PAHs should be carried out in winter.

ARTICLE INFO

ABSTRACT

The seasonal differences of the polycyclic aromatic hydrocarbons (PAHs) content in lichen transplants were investigated in an area of ca. 40 square kilometers in NE Italy characterized by different land use. Two sets of samples of the epiphytic lichen *Pseudevernia furfuracea* were collected in a pristine area of the Carnic Alps and transplanted to 40 exposure sites for a period of two months, respectively in late winter and in summer. Results revealed a pronounced difference between the two seasons in terms of PAH content and distribution patterns. After the summer exposure the PAH concentrations in the transplants were more than one order of magnitude lower than after the winter exposure (ranging from 48.22 to 272.73 ng g^{-1} dw and from 289.73 to 1575.85 ng g^{-1} dw in the summer and winter samples respectively). Also the main emission sources changed, mostly due to the drastic reduction in the emissions by wood burning for domestic heating and to the different meteorological conditions. In summer PAHs degradation was enhanced by intense UV radiation, high temperatures, and presence of ozone. The implications of these findings for the biomonitoring of PAHs pollution are addressed.

© 2015 Elsevier Ltd. All rights reserved.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental pollutants that originate mainly from anthropogenic sources such as industrial activities, road traffic, and domestic heating through processes of incomplete combustion of biomass and fossil fuels (Harvey, 1991). They have received much attention in the last decades due to their mutagenic and carcinogenic properties (Augusto et al., 2012; Boström et al., 2002) with tendency to bioaccumulate and persist in the environment (Augusto et al., 2013a). Once emitted, they will disperse in the atmosphere and travel for long distances depending on their weight and on air temperature (Beyer et al., 2003), undergoing photochemical and chemical degradation (Alves, 2008; Boström et al., 2002; Brown and Brown, 2012; Jung et al., 2010; Schauer et al., 2003).

Lichens, a symbiotic association between fungi and algae and/or cyanobacteria, unlike plants, lack a cuticle layer that limits the inflow of pollutants and thus absorb contaminants and nutrients from both wet and dry atmospheric deposition (Bargagli, 1998;

---

* Corresponding author.
E-mail addresses: dkodnik@units.it (D. Kodnik), fcandotto@units.it (F. Candotto Carniel), slicen@units.it (S. Licen), arianna.tolloi@phd.units.it (A. Tolloi), barbierp@units.it (P. Barbieri), tretiach@units.it (M. Tretiach).
[1] Present address: University of Innsbruck, Institute of Botany, Sternwartestraße 15, A-6020 Innsbruck, Austria.

Garty, 2000). They are among the best and most frequently used biomonitors of air-borne pollutants, so that a norm for their use has just been edited by the European Committee for Standardization (CEN, EN 54 16413:2014). Lichens are valid biomonitors of PAHs because (*i*) they bioaccumulate both vapor- and solid-phase PAHs (Augusto et al., 2013a; Guidotti et al., 2003, 2009; Nascimbene et al., 2014; Protano et al., 2014; Shukla and Upreti, 2009; Van der Wat and Forbes, 2015); (*ii*) they show a "memory" effect with a retro-active period of 45–60 days, and correlations can be made between the concentrations found in lichens and in the air (Augusto et al., 2013b); (*iii*) the low costs of material recovery allow to apply a high sampling density that improves the experimental design of field investigations; (*iv*) by using transplants, it is also possible to cover those areas where autochthonous macrolichens are frequently absent (e.g. urban centers); (*v*) based on the PAH profile it is possible to identify the source(s) of emission (forest areas are dominated by 3-ring PAHs, urban by 4-ring PAHs, industrial pyrogenic by 5- and 6-ring PAHs and industrial petrogenic by 2-ring PAHs, see Augusto et al., 2009; Shukla et al., 2012).

However, the extent of the seasonal variation in the PAHs content of lichens is largely unknown. Several physical-chemical and biological factors certainly interplay to determine a multi-faceted figure. Lichen growth forms (Blasco et al., 2011), age (Bargagli et al., 1987), lipid content (Zhu et al., 2015), development of reproductive structures (Adamo et al., 2007) are all factors that may influence the bioaccumulation capability. This is further complicated by the variable amounts of PAHs released into the environment, and by the different fate in the atmosphere of e.g. 2- and 6-ring PAHs.

The objective of this study was to verify the seasonal differences of the PAH content in lichen transplants in a mixed land use area of NE Italy selected in order to have:

- all the primary PAH emission sources (different industrial activities, road traffic and domestic heating);
- to evaluate the effects of the different meteorological conditions on the ability of lichens to accumulate PAHs;
- to observe if and to what extent the PAH distribution changes with location between the seasons.

2. Materials and methods

2.1. Study area

The study area is located at the foot of the Carnic pre-Alps (NE Italy; 46°10′N 12°44′E; Fig. 1), at an altitude of 200–380 m a.s.l. It covers almost 40 km^2 and hosts a resident population of ca. 16,240 inhabitants. It was selected in order to include different land uses: natural, agricultural, urban, and industrial, intermingled to form the typical mixed land use patchwork that extends over large areas of N Italy. The main potential pollution sources of the study area are: (*i*) an isolated, medium-sized cement plant (clinker production: 556,000 ton year^{-1} in 2012; M. Vicenzetto, personal communication); (*ii*) a large industrial zone that hosts two steel works, several knife manufacturing factories and a chemical plant producing pesticides; (*iii*) vehicular traffic, concentrated in the main urban centers and along a national road ("464 – Spilimbergo") that crosses the northern part of the study area; (*iv*) domestic heating emissions.

In the study area 37 exposure sites were located at the intersections of a 700 m step grid, and 3 further sites were located in the nearby urban centers (Fig. 1). Following the Corine Land Cover (2006) classification, 21 sites belonged to the agricultural stratum, 10 to the urban stratum, 5 to the industrial stratum and 4 to the

forest stratum. Detailed information about the exposure sites is listed in Table SD-1 in the Supplementary Data.

The data on the meteorological conditions during the exposure periods was retrieved from the closest weather station, that is located outside Arba (Fig. 1), and is run by the Civil Protection Service.

2.2. Lichen sampling, exposure and recovery

The epiphytic lichen *Pseudevernia furfuracea* (L.) Zopf. var. *furfuracea* was selected because of its commonness, good resistance to transplantation (Tretiach et al., 2007), and frequent use as biomonitor of trace elements and PAHs (Table SD-2 in the Supplementary Data). Samples were collected in the Carnic Alps (Casera Razzo, Belluno; 46°25′N 12°44′E) at 1700 m a.s.l., far from local anthropogenic sources (Adamo et al., 2008; Tretiach et al., 2011), from branches at 2–4 m above ground of solitary larch (*Larix decidua* Mill.) trees, in two distinct periods: 9th January 2012 for the late winter exposure (see below), and 8th June 2012 for the summer exposure (see below). The material was collected along with ca. 15–20 cm long piece of the supporting twig using garden shears. The material was transported to the laboratory in paper bags and left to dry out in dim light at room temperature. Moderately isidiate samples were selected avoiding those over-isidiate or with apothecia (because these structures may alter the surface/weight ratio), infected by lichenicolous fungi or covered by epiphytic algae (Tretiach et al., 2007, 2011).

One twig carrying 1–3 thalli was attached to a 120 cm long bamboo stick using plastic bonds (both first rinsed with distilled water) and sealed in plastic bags until exposure to avoid contamination.

Two exposure periods were selected, one in late winter (15th February–17th April 2012), and the other in summer (19th June-21st August 2012), using two different sets of samples. At each site two bamboo sticks were attached to the external branches of deciduous trees at approximately 4 m above ground using plastic bonds. In both cases, the exposure was done within 12 h of field work. After the two-month exposure the samples were retrieved with a piece of the bamboo stick, sealed in plastic bags, and transported in a coolbag within 6 h to the laboratory, where the bags were opened, and the samples were left to dry out at room temperature for 12 h. 600 mg of ca. 2.5 cm long lobe portions were selected for each sample, sealed in glass cans and kept in the dark at −20 °C until analysis minimizing potential losses of PAHs from samples (Augusto et al., 2013b). Samples of the site 4B were lost during the winter exposure and samples of the sites 1E, 2C and 5B were lost during the summer exposure.

2.3. PAHs analyses

The analysis procedure is based on the protocol of Domeño et al. (2006), slightly modified as follows.

Before extraction the samples were allowed to defrost and then finely chopped with scissors. The extraction was performed using an Accelerated Solvent Extractor (ASE X-100, Dionex): ca. 600 mg of dried sample was placed in the extractor cell together with 600 mg of dried Na_2SO_4 and 600 mg of Florisil. A solution of surrogate standards of deuterated PAHs (100 μL, 1.2 μg mL^{-1} Wellington L429-IS) was added for the evaluation of recovery rates. The extraction was performed in a mixture of dichloromethane/acetone 1:1 for trace analysis (Pestanal, Sigma–Aldrich) at 140 °C and 100 atm (3 extraction cycles of 10 min). The extract (ca. 120 mL) was rotary evaporated under reduced pressure to ca. 1 mL. The reduced extract was purified by column chromatography on dried alumina activated at 400 °C for 6 h, eluting with dichloromethane. The
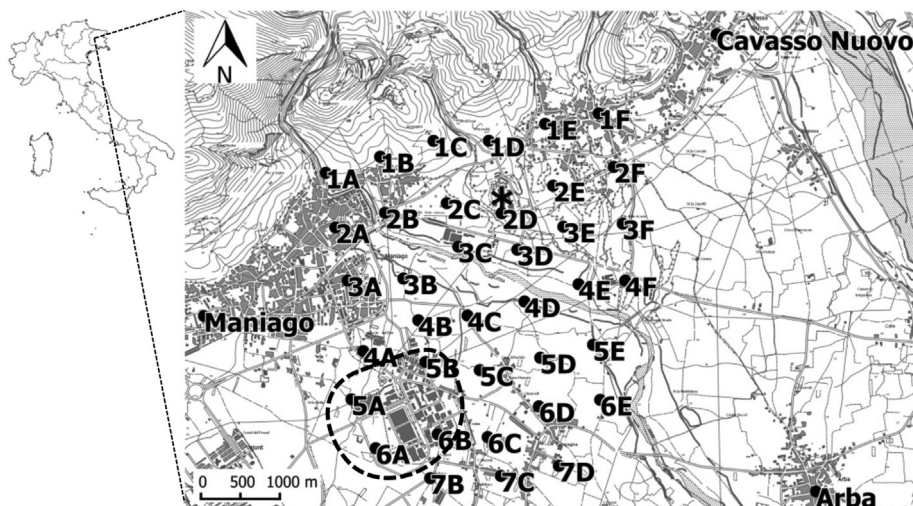
Fig. 1. Study area with location of the 40 exposure sites. The cement plant is indicated by an asterisk, the industrial area by a closed dashed line.

eluate was evaporated to dryness by a gentle nitrogen stream and the residue dissolved in approximately 2 mL of cyclohexane for trace analysis (Pestanal, Sigma—Aldrich). An exact amount of Pyrene-D (100 μL of a 20 μg mL^{-1} solution, Aldrich 490695) was added as internal standard to every extracted solution in order to avoid bringing the final volume exactly up to 2 mL.

The analysis was carried out by a GC—MS system (Agilent 6890/5973 Inert, Agilent DB 5MS UI capillary column 30 m × 0.25 mm i.d. × 0.25 μm film thickness) with helium as carrier gas. The system was equipped with an autosampler (Gerstel MPS2).

The GC oven temperature program started at 55 °C then was ramped to 200 °C at 25 °C/min, to 320 °C at 10 °C/min and to 325 at 25 °C/min, with a final isothermal stage held for 10 min. The mass spectrometer operated in selective ion monitoring (SIM) mode. The following mass ions were used for the quantification of individual PAHs: fluoranthene (Fth, m/z 202), pyrene (Pyr, m/z 202), benzo[a]anthracene (BaA, m/z 228), chrysene (Chr, m/z 228), benzo[b]fluoranthene (BbF, m/z 252), benzo[k]fluoranthene (BkF, m/z 252), benzo[a]pyrene (BaP, m/z 252), indeno[1,2,3-cd]pyrene (Ind, m/z 276), dibenzo[a,h]anthracene (DahA, m/z 278), and benzo[g,h,i]perylene (BghiP, m/z 276). The PAH recoveries were calculated respectively by fluoranthene-d10 (m/z 212, for Fth and Pyr), benzo[a]anthracene-d12 (m/z 240, for BaA and Chr), benzo[b]fluoranthene-d12 (m/z 264, for BbF and BkF), benzo[a]pyrene-d12 (m/z 264, for BaP), indeno[1,2,3-cd]pyrene-d12 (m/z 288, for Ind and DahA), benzo[g,h,i]perylene-d12 (m/z 288, for BghiP). Six calibration solutions with concentrations ranging from 5 ng mL^{-1} to 100 ng mL^{-1} for each PAH were prepared in triplicate by appropriate dilution of the certified PAHs standard mixture (PAH-EPA 610 by Sigma—Aldrich) in cyclohexane for trace analysis (Pestanal, Sigma—Aldrich) in order to quantify the PAH concentrations. A mid-range calibration standard solution was re-measured every six samples in order to ensure that the instrument was still calibrated.

In the absence of a certified lichen standard material, instrumental signal to noise ratio was calculated on a 10 ng mL^{-1} standard solution in order to determine the limit of detection (LOD) and the limit of quantification (LOQ). LOD is determined as the amount of analyte that generates a signal that is three times the noise in regions of the chromatogram close to the elution time of the peak of interest, that are approximately twenty times the analyte peak width. LOQ is determined as the amount of analyte that generates a signal that is ten times the noise. LODs (expressed in ng g^{-1} of PAHs

in a theoretical 600 mg lichen dw) were respectively: 0.10 (Fth), 0.14 (Pyr), 0.19 (BaA), 0.22 (Chr), 0.48 (B(b + k)F), 0.36 (BaP), 0.18 (Ind), 0.27 (DahA), 0.30 (BghiP).

2.4. Statistics

Descriptive statistics were performed using Microsoft Excel (Microsoft Office Professional Plus 2010). Box-plots, principal component analysis (PCA) and Mann—Whitney U Test were performed to verify differences between groups using STATISTICA 8.0 StatSoft Inc. (2007). For samples in which a compound was under the limit of detection (LOD) of the instrument its concentration was assumed to be the LOD value. The maps representing the distribution patterns of the total PAHs were calculated using Surfer (Win32) 6.04 (Golden Software Inc.) with Inverse Distance to a Power as the gridding method and a medium Smooth Contour for the creation of the maps, the intervals between the minimum and maximum concentration values have been divided into 10 classes of equal extent. The equivalent toxicity quotient (TEQ) of the PAHs was calculated according to the toxic equivalency factors proposed by Nisbet and LaGoy (1992). The localization of the exposure sites in the study area was graphically represented using Quantum GIS 1.8.0.-Lisboa (2012).

3. Results

3.1. Meteorological conditions

The main climatic differences observed during the two exposure periods are summarized in Fig. 2. The total rainfall was similar, 202.2 mm in winter and 213.8 mm in summer. The rain events of the winter exposure were concentrated at the end of the period, while those of the summer exposure were more evenly distributed but much briefer and significantly more intense (p = 0.001; Fig. 2a). The atmospheric pressure was significantly higher during the winter period (p = 0.009; Fig. 2b). The winds were mainly north-northwesterly all year round (Fig. 2c). The mean temperature was 10.0 °C during the winter exposure, ranging from 5.6 to 15.1 °C (mean daily minimum and maximum, respectively) and 24.6 °C during the summer exposure, ranging from 19.3 to 30.2 °C (Fig. 2d).
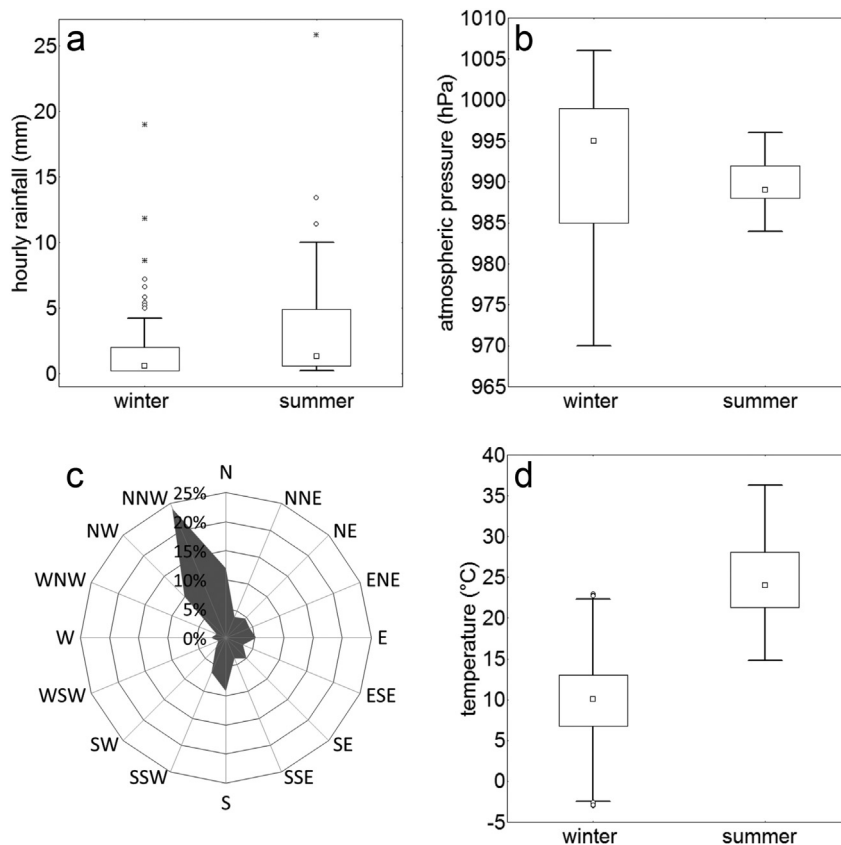
Fig. 2. Hourly rainfall (a), atmospheric pressure (b), overall wind direction (c), and air temperature (d) in the study area of Fig. 1 during the two exposure periods, winter and summer (□: median; box: 25–75%; whiskers: non outlier ranges; ○: outliers; ∗: extremes).

3.2. PAH content

In total, 10 PAHs were detected: fluoranthene (Fth), pyrene (Pyr), benzo[a]anthracene (BaA), chrysene (Chr), benzo[b]fluoranthene and benzo[k]fluoranthene being treated together as benzo[b + k] fluoranthene (B(b + k)F) due to the difficulty in clearly discerning among their peaks, benzo[a]pyrene (BaP), indeno[1,2,3-c,d]pyrene (Ind), benzo[g,h,i]perylene (BghiP) and dibenzo[a,h]anthracene (DahA). Lighter (two and three ring) PAHs — with higher volatilities, smaller octanol-air partitioning and more prone to volatilization from lichens (Simonich and Hites, 1994; Odabasi et al., 2006; Schrlau et al., 2011) — were not considered for analytical quantification due to both unfavorable temperature dependent lichen-air partitioning in the environment and non-relevant human toxicity in comparison with heavier congeners (Boström et al., 2002; IARC WG, 2010). Moreover, our purification procedure, while guaranteeing adequate recoveries for heavier PAHs, does not allow the removal of certain lichen substances eluting at retention times similar to those of two and three ring PAHs and thus generating undesired noise.

The sum of the concentrations of the 10 PAHs in the pre-exposure winter samples ranged from 65.83 to 211.92 ng g^{-1} dw (Table 1, Table SD-3), and were significantly lower than in all the post-exposure winter samples. The concentrations in the latter ranged in fact from 289.73 to 1575.85 ng g^{-1} dw (Table 1, Table SD-3), with BaP under the LOD of the instrument in only one sample, Ind in two samples, BghiP in 53.8% and DahA in 69.2% of the samples. In these samples the concentrations of 5-, 6-ring PAHs, as well as of BaP, were rather low and equally distributed in the study area with no significant differences between land-use strata,

whereas 95% of the total PAH content was given by 4-ring PAHs (Fig. 3). The significantly lowest concentrations of 4-ring PAHs were found in the forest stratum (Table 2, Fig. 4a), followed by the industrial and agricultural strata (with similar values), while significantly higher concentrations were found in the urban stratum, although the TEQ values denote a low carcinogenicity risk (Fig. 5, Table SD-5).

The concentrations in the pre-exposure summer samples were somewhat lower than in the winter ones, ranging from 65.43 to 130.07 ng g^{-1} dw (Table 1, Table SD-4). The concentrations in the post-exposure summer transplants were ca. an order of magnitude lower than in winter, ranging from 48.22 to 272.73 ng g^{-1} dw (Table 1, Table SD-4). Several PAHs were often under the LOD of the instrument: B(b + k)F in 29.7% of the samples, BaP in 75.7%, Ind in 86.5%, BghiP in 94.6% and DahA in all the samples. The post-exposure concentrations in the summer samples of both forest and agricultural strata were actually significantly lower than in the pre-exposure samples, while the post-exposure samples of the urban and industrial strata were not significantly different from the pre-exposure samples. In this season as well there were no significant differences between land-use strata in terms of concentrations of the 5-, 6-ring PAHs and BaP, whereas 95% of the total PAH content was given by 4-ring PAHs (Fig. 3). The significantly lowest concentrations of 4-ring PAHs were found in the forest and agricultural strata (which had similar values), while the significantly highest concentrations were found in the urban and industrial strata (also with similar values between them: Table 2, Fig. 4b).

Between the winter and the summer exposure not only a pronounced difference in PAHs concentrations of post-exposure samples was observed, but also an entirely different correlation

Table 1

PAHs content (ng g^{-1} dw) measured in pre- and post-exposure samples of the lichen *Pseudevernia furfuracea* transplanted in the sites of Fig. 1 subdivided according to land use.

Winter		Pre-exposure	Post-exposure			
Land use strata			Agricultural	Forest	Industrial	Urban
No. of sites			20	4	5	10
4-ring PAHs	Mean	156.87	586.59	314.58	702.89	1015.36
	SD	44.18	190.16	84.59	146.47	268.17
	Min	55.54	403.62	236.77	509.36	691.06
	Max	199.17	1138.41	430.59	905.61	1546.89
5-ring PAHs	Mean	14.86	23.84	32.48	21.66	31.77
	SD	6.8	7.05	9.29	7.65	21.58
	Min	3.55	13.68	20.38	13.33	19.8
	Max	22.12	44.16	41.68	34.01	92.49
6-ring PAHs	Mean	1.77	7.49	23.56	4.79	8.03
	SD	1.32	5.05	23.99	1.87	4.54
	Min	0.71	0.71	3.26	2.25	3.54
	Max	4.6	19.75	58.32	6.68	19.7
Total PAHs	Mean	173.5	617.91	370.62	729.34	1055.16
	SD	45.18	190.83	85.03	154.14	270.4
	Min	65.83	445.51	289.73	526.25	724.8
	Max	211.92	1175.59	464.35	944.75	1575.85
Summer		Pre-exposure	Post-exposure			
Land use strata			Agricultural	Forest	Industrial	Urban
No. of sites			20	4	4	9
4-ring PAHs	Mean	87.12	81.12	57.89	138.97	99.34
	SD	16.12	47.34	8.2	69.32	21.02
	Min	55.54	46.88	45.99	94.06	66.79
	Max	109.29	271.39	63.54	242.23	132.03
5-ring PAHs	Mean	7.48	3.56	3.53	11.35	6.12
	SD	4.84	2.4	3.35	9.31	4.9
	Min	4.74	0.63	0.63	0.63	0.63
	Max	20.06	9.31	6.45	23.35	15.76
6-ring PAHs	Mean	0.92	1.05	0.71	0.71	1.78
	SD	0.62	0.86	0	0	2.17
	Min	0.71	0.71	0.71	0.71	0.71
	Max	2.58	3.74	0.71	0.71	6.42
Total PAHs	Mean	95.51	85.73	62.14	151.03	107.24
	SD	18.47	47.19	7.35	61.82	18.47
	Min	65.43	48.22	53.15	116.86	75.79
	Max	130.07	272.73	70.66	243.57	133.37
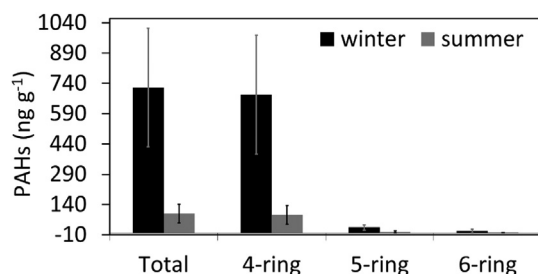
Fig. 3. Average and standard deviation of total, 4-, 5- and 6-ring PAHs content (ng g^{-1} dw) in the winter and summer samples of the lichen *Pseudevernia furfuracea* exposed in the 40 sites of Fig. 1.

Table 2

p-values (Mann-–Whitney *U* test) of the differences between the 4-ring PAH concentrations measured in post-exposure samples of the lichen *Pseudevernia furfuracea* exposed in the sites of Fig. 1 subdivided according to land use. (p-value considered significant <0.05).

Winter 4-ring PAHs	Forest	Industrial	Urban
Agricultural	**0.001**	0.097	**0**
Forest	–	**0.016**	**0.002**
Industrial	–	–	**0.040**
Summer 4-ring PAHs	Forest	Industrial	Urban
Agricultural	0.157	**0.005**	**0.007**
Forest	–	**0.029**	**0.003**
Industrial	–	–	0.260

The values that are marked in bold are the ones considered significant (p < 0.05).

between PAHs and land use strata (Fig. 6).

In the winter campaign (Fig. 6a,b) the urban sites were distinctly separated from all the other sites at one end of the first component and were strongly associated to the 4-ring PAHs Fth, Pyr, BaA and Chr. The 5- and 6-ring PAHs, as already mentioned, did not have a particular association with any stratum but only with 2 samples. The forest site 1B had high concentrations of 6-ring PAHs, and the urban site 4A had a high concentration of B(b + k)F, the possible emission sources remaining unidentified (Fig. 1).

In the summer campaign (Fig. 6c,d) the differences between strata were much less pronounced, although the industrial sites were those more strongly associated with 4-ring PAHs, followed by the urban sites, while the agricultural and forest sites mostly overlapped. The 6-ring PAHs seemed to have a strong association with two urban sites (Cavasso Nuovo and Maniago; Fig. 1), due to the fact that their 6-ring PAHs content was above the LOD (actually, being the only two in the case of BghiP).

As an example of the differences between the two exposure periods, Fig. 7 shows the distributional patterns of the total PAHs load drawn with Surfer (Win32) 6.04 (Golden Software Inc.).
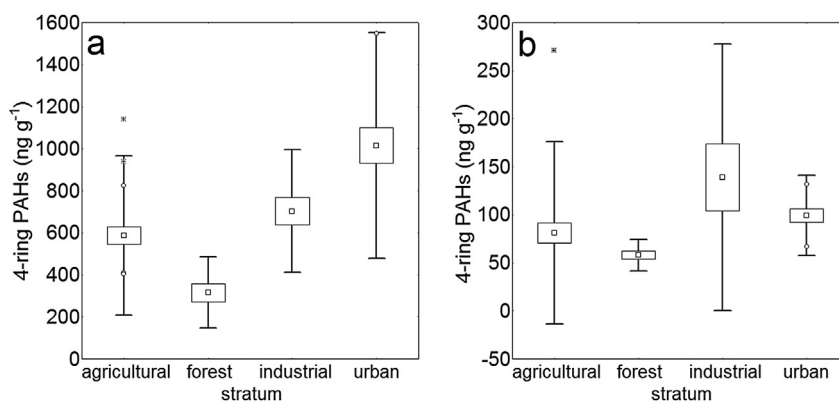
Fig. 4. 4-ring PAHs content (ng g^{-1} dw) in the samples of the lichen *Pseudevernia furfuracea* exposed in winter (a) and in summer (b) in the 40 sites of Fig. 1 subdivided according to land use (□: mean; box: mean ± SE; whiskers: mean ± 2 × SD; ○: outliers; *: extremes).
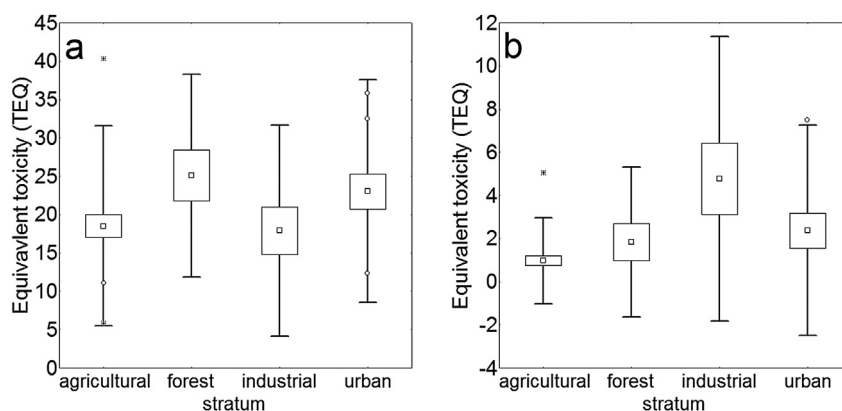

Fig. 5. Equivalent Toxicity (TEQ) values of PAHs measured in the samples of the lichen *Pseudevernia furfuracea* exposed in winter (a) and in summer (b) in the 40 sites of Fig. 1 subdivided according to land use (□: mean; box: mean ± SE; whiskers: mean ± 2 × SD; ○: outliers; *: extremes).

4. Discussion

This study shows that the exposure period of two months was sufficient for transplants of the lichen *Pseudevernia furfuracea* (*i*) to accumulate PAHs, and (*ii*) to reveal specific distribution patterns on a highly heterogeneous territory. The total PAH content measured after both exposure periods (Table 1) is within the ranges reported in the literature (Table SD-2), although this comparison is problematic, since (*i*) the species may differ in PAH accumulation rates (Blasco et al., 2011), which mostly depends on their surface/weight ratio (Adamo et al., 2007; Bertuzzi and Tretiach, 2013), and (*ii*) the analytical procedures differ from study to study, and this might deeply influence the results (Domeño et al., 2006). The most striking result, however, is the confirmation of a pronounced seasonal difference in the transplant PAHs accumulation, with winter-exposed samples one order of magnitude more heavily enriched than summer-exposed ones, and with 4-ring PAHs apparently more variable than 5- and 6-ring PAHs. This is the consequence of significant differences between the two exposure periods in terms of sources, emission levels, climatic conditions and PAHs degradation phenomena.

It is unlikely that the local industries (the most important being the two steel works and the cement plant) could be the sources of the 4-ring PAHs. In the study area the prevailing wind direction is relatively constant throughout the year, being mostly north-northwesterly (Fig. 2c), and this is in contradiction with the location of the steel works (in the southwestern corner of the study area, see Fig. 1) and the cement plant (approximately at the center of the study area, see Fig. 1), on the one side, and with the location of the most heavily polluted exposure sites, on the other side. The latter belong in fact to the urban stratum, and are all located in the north-western corner of the study area, or in the two small villages of Cavasso Nuovo and Arba, outside the grid (Fig. 1). Interestingly, the clinker production itself, that is the core of the industrial processes of a cement plant, is considered to be PAHs-free, due to the long retention times (over 2 s), the high temperatures (1400–1500 °C), and the strongly oxidative atmosphere of the rotary kiln (Mokrzycki and Uliasz-Bocheńczyk, 2003). These favorable conditions even allow the use of refuse-derived fuel (RDF; mainly plastics and biodegradable waste) instead of coal, methane, etc. (European Cement Association CEMBUREAU).

Vehicular traffic may also generate 4-ring PAHs due to incomplete fuel combustion. However, no evidence is available to substantiate a significant difference between the winter and summer traffic load in the study area, and therefore the contribution of this source to the overall 4-ring PAHs load must be considered as negligible. By contrast, the most probable source of 4-ring PAHs is domestic heating, and particularly wood burning. This activity, being based on a renewable source, is perceived as "green" and of low environmental impact. Actually, due to the low combustion temperatures and the poor mixing efficiencies of oxygen and fuel, wood burning in traditional fireplaces and ovens is an important source of organic compounds released into the environment (Mastral and Callén, 2000). The wood combustion generates high
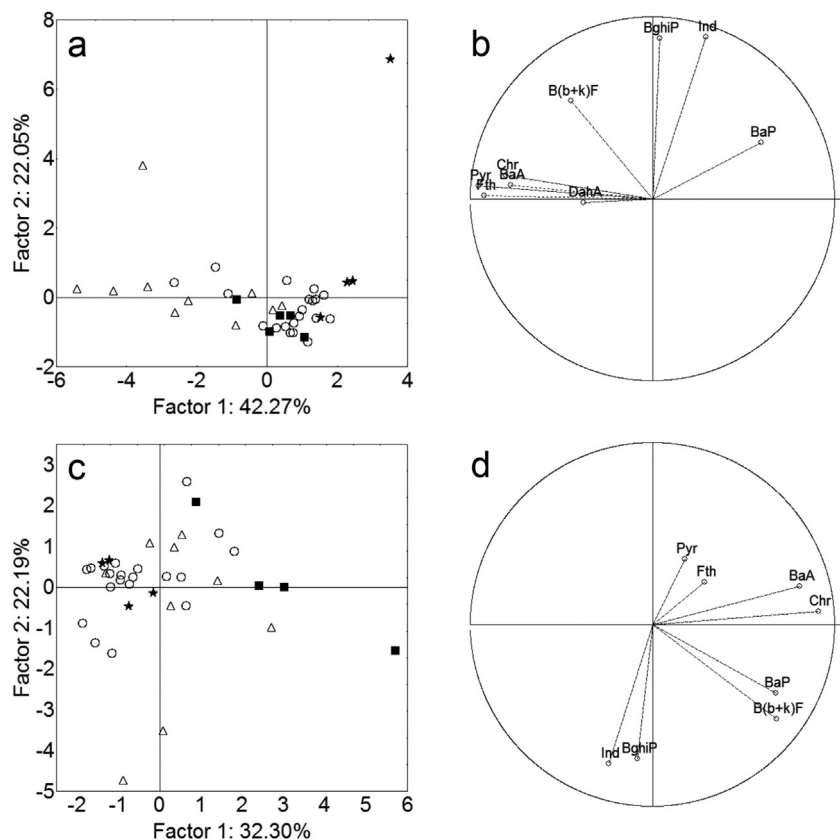
Fig. 6. Principal component analysis (PCA) of the samples of the lichen *Pseudevernia furfuracea* exposed in winter (a) and in summer (c) in the 40 sites of Fig. 1 classified according to land use (○ = agricultural; ★ = forest; ■ = industrial; Δ = urban), and the corresponding loading plots of PAHs identified in the samples (b; d). DahA was eliminated from the summer analysis because it was below LOD in all the samples.
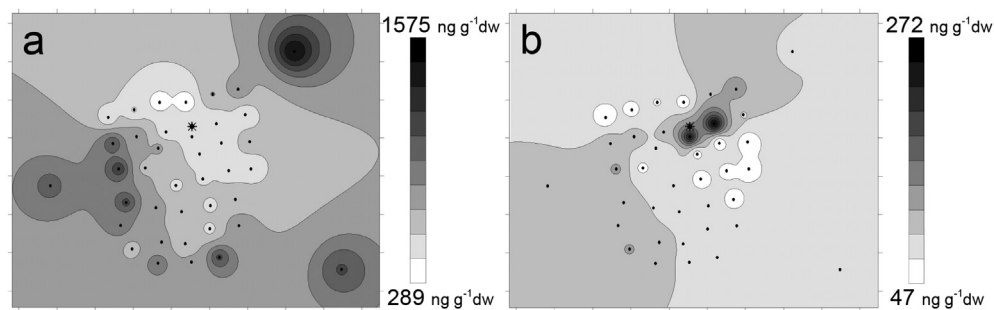

Fig. 7. Distribution patterns of the total PAHs load in the study area of Fig. 1 based on the concentrations measured in the samples of the lichen *Pseudevernia furfuracea* exposed in winter (a) and in summer (b).

levels of fluoranthene, pyrene, benzo[a]anthracene and chrysene (Boström et al., 2002; Singh et al., 2013), which are the dominant compounds of our urban winter samples (Fig. 6a,b), luckily with low carcinogenic potency (Nisbet and LaGoy, 1992). Two observations support this conclusion: (*i*) wood burning is quite frequent in the study area, with more than 25% of the households that use it for domestic heating, while the use of wood burning for cooking is limited to sporadic professional activities, and (*ii*) domestic heating is restricted by law to the winter period (data from ARPA FVG, 2013a). This automatically brings down the emissions in the summer period, with the high temperatures that may further reduce the semi-volatile 4-ring PAHs eventually accumulated in the lichen samples (Beyer et al., 2003; Panther et al., 1999; Singh et al., 2013).

As far as the sources of the 5- and 6-ring PAHs are concerned,

their low concentrations and uniform distribution after both exposures suggest that in the study area there are no important industrial pyrogenic pollution sources (Augusto et al., 2009; Shukla et al., 2012). However, in summer, the post-exposure concentrations of the 5- and 6-ring PAHs, scarcely volatile in comparison to the 4-ring PAHs, were even lower than the pre-exposure ones. This might be due to the short but intense thunderstorms that occurred in the study area in summer (Fig. 2a). These can have a much stronger wash effect than the prolonged but lighter rains of the winter period on the particulate matter deposited on the sample surface, and thus on the 5- and 6-ring PAHs that are associated with it (Augusto et al., 2013a, 2013b). A second explanation − and perhaps more robust than the previous one − is certainly the photochemical and chemical degradation to which all PAHs are

subjected when exposed to UV (Boström et al., 2002; Wild et al., 2005), although 5- and 6-ring PAHs less intensely than lighter PAHs (Nadal et al., 2006). Also oxidative molecules such as ozone and similarly reactive trace gases may interfere (Alves, 2008; Brown and Brown, 2012). Ozone tends to form in presence of NO_x (mostly derived from combustion processes) when there are high temperatures and intense UV radiation, conditions that at middle latitudes are typically present in summer but not in winter (Lorenzini and Nali, 2005). Schauer et al. (2003) demonstrated that filter sampling carried out without the use of a diffusion denuder that removes ozone can lead to an underestimation of the actual PAHs content in the air by up to 100% or even more due to oxidative degradation. Lichens lack the cuticle wax layer that characterizes the leaves of vascular plants. This certainly favors the accumulation of air-borne contaminants (Augusto et al., 2010; Schrlau et al., 2011), but it also permits the diffusion of ozone towards the inner layers (Pellegrini et al., 2014). We can thus assume that the degradation that occurs on aerosol filters may occur in lichens as well, causing a potentially significant underestimation of the real PAHs emission levels in the study area during the summer season, when the ozone levels are usually the highest (Lorenzini and Nali, 2005). Interestingly, when this study was carried out, the ozone levels in the study area exceeded the limit of 120 µg m^{-3} (EU Directive 2008/50/CE) more than 25 times, with peaks that ranged between 180 and 192 µg m^{-3} (ARPA FVG, 2009, 2013b).

5. Conclusions

There are important seasonal differences in the environmental PAHs intercepted by lichen transplants in the selected study area. These changes may depend on modifications in emission intensity, and on environmental conditions that interfere with both kinetics and dynamics of transport and deposition, and with the successive degradation processes of PAHs that are promoted by high temperatures, UV and oxidative conditions, consequently the entity of the changes may differ at other locations with different environmental conditions.

The seasonal differences existing in the PAHs loads should be taken into account when planning a biomonitoring survey. Studies performed during the warmest season, in fact, may correctly describe the distribution patterns of these pollutants over the territory, but they certainly underestimate the potential PAHs load, that typically occurs in the coldest periods of the year. By contrast, studies based on samples collected in different seasons are likely to over-estimate the variation in emission intensity of the purported sources, because some of the variation is actually due to differences in the climatic conditions that favor the interception/discharge of PAHs-enriched particles or the PAHs degradation processes.

At middle latitudes, the winter period might thus be favorable to detect high PAH levels, because photo- and thermal degradation is low, and more emission sources are potentially active, whereas in the summer period it might be preferable to monitor the products derived from PAHs oxidation (e.g. epoxide, endoperoxide and hydroxy species, quinones, diones etc., see Chu et al., 2010).

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://

dx.doi.org/10.1016/j.atmosenv.2015.04.067.

References

Adamo, P., Agnorelli, C., Bargagli, R., Giordano, S., Modenesi, P., Pittao, E., Tretiach, M., 2007. Lichen and moss bags as monitoring devices in urban areas. Part II: trace element content in living and dead biomonitors and comparison with synthetic materials. Environ. Pollut. 146, 392–399.

Adamo, P., Bargagli, R., Giordano, S., Modenesi, P., Monaci, F., Pittao, E., Spagnuolo, V., Tretiach, M., 2008. Natural and pre-treatments induced variability in the chemical composition and morphology of lichens and mosses selected for active monitoring of airborne elements. Environ. Pollut. 152, 11–19.

Alves, C.A., 2008. Characterization of solvent extractable organic constituents in atmospheric particulate matter: an overview. Ann. Braz. Acad. Sci. 80, 21–82.

ARPA FVG, 2009. Piano regionale di miglioramento della qualità dell'aria. Palmanova, Italy.

ARPA FVG, 2013a. Distribuzione dei vettori energetici in Friuli Venezia Giulia per il riscaldamento domestico e loro emissioni. Palmanova, Italy.

ARPA FVG, 2013b. Relazione sulla qualità dell'aria nella regione Friuli Venezia Giulia, anno 2012. Palmanova, Italy.

Augusto, S., Máguas, C., Branquinho, C., 2013a. Guidelines for biomonitoring persistent organic pollutants (POPs), using lichens and aquatic mosses – a review. Environ. Pollut. 180, 330–338.

Augusto, S., Máguas, C., Matos, J., Pereira, M.J., Branquinho, C., 2010. Lichens as an integrating tool for monitoring PAH atmospheric deposition: a comparison with soil, air and pine needles. Environ. Pollut. 158, 483–489.

Augusto, S., Máguas, C., Matos, J., Pereira, M.J., Soares, A., Branquinho, C., 2009. Spatial modeling of PAHs in lichens for fingerprinting of multisource atmospheric pollution. Environ. Sci. Technol. 43, 7762–7769.

Augusto, S., Pereira, M.J., Máguas, C., Branquinho, C., 2013b. A step towards the use of biomonitors as estimators of atmospheric PAHs for regulatory purposes. Chemosphere 92, 626–632.

Augusto, S., Pereira, M.J., Máguas, C., Soares, A., Branquinho, C., 2012. Assessing human exposure to polycyclic aromatic hydrocarbons (PAH) in a petrochemical region utilizing data from environmental biomonitors. J. Toxicol. Environ. Health Part A 75, 819–830.

Bargagli, R., 1998. Lichens as biomonitors of airborne trace elements. In: Bargagli, R. (Ed.), Trace Elements in Terrestrial Plants: an Ecophysiological Approach to Biomonitoring and Biorecovery. Springer-Verlag Berlin Heidelberg, New York, pp. 179–206.

Bargagli, R., Iosco, F.P., D'Amato, M.L., 1987. Zonation of trace metal accumulation in three species of epiphytic lichens belonging to the genus *Parmelia*. Cryptogam. Bryol. Lichénol. 8, 331–337.

Bertuzzi, S., Tretiach, M., 2013. Hydrogen sulphide inhibits PSII of lichen photobionts. Lichenol. 45, 101–113.

Beyer, A., Wania, F., Gouin, T., Mackay, D., Matthies, M., 2003. Temperature dependence of the characteristic travel distance. Environ. Sci. Technol. 37, 766–771.

Blasco, M., Domeño, C., López, P., Nerín, C., 2011. Behaviour of different lichen species as biomonitors of air pollution by PAHs in natural ecosystems. J. Environ. Monit. 13, 2588–2596.

Boström, C.E., Gerde, P., Hanberg, A., Jernström, B., Johansson, C., Kyrklund, T., Rannug, A., Törnqvist, M., Victorin, K., Westerholm, R., 2002. Cancer risk assessment, indicators, and guidelines for polycyclic aromatic hydrocarbons in the ambient air. Environ. Health Perspect. 110, 451–489.

Brown, R.J.C., Brown, A.S., 2012. The Effect of Degradation by Gaseous Oxidants on Measured Benzo[a]pyrene Concentrations. NPL Report AS 72. National Physical Laboratory, Teddington, Middlesex.

Chu, S.N., Sands, S., Tomasik, M.R., Lee, P.S., McNeill, V.F., 2010. Ozone oxidation of surface-adsorbed polycyclic aromatic hydrocarbons: role of PAH-surface interaction. J. Am. Chem. Soc. 132, 15968–15975.

Domeño, C., Blasco, M., Sánchez, C., Nerín, C., 2006. A fast extraction technique for extracting polycyclic aromatic hydrocarbons (PAHs) from lichens samples used as biomonitors of air pollution: dynamic sonication versus other methods. Anal. Chim. Acta 569, 103–112.

European Cement Association CEMBUREAU: http://www.cembureau.be/topics/resource-efficiency-cement-industry/co-processing-cement-industry-using-waste-resource, last visit: 22th August 2014.

Garty, J., 2000. Environmental and element content in lichens. In: Markert, B., Friese, K. (Eds.), Trace Elements – their Distribution and Effects in the Environment. Elsevier Science, Amsterdam, pp. 245–276.

Guidotti, M., Stella, D., Dominici, C., Blasi, G., Owczarek, M., Vitali, M., Protano, C., 2009. Monitoring of traffic-related pollution in a province of central Italy with transplanted lichen *Pseudevernia furfuracea*. Bull. Environ. Contam. Toxicol. 83, 852–858.

Guidotti, M., Stella, D., Owczarek, M., De Marco, A., De Simone, C., 2003. Lichens as polycyclic aromatic hydrocarbon bioaccumulators used in atmospheric pollution studies. J. Chromatogr. A 985, 185–190.

Harvey, R.G., 1991. Environmental occurrence and cancer. In: Harvey, R.G. (Ed.), Polycyclic Aromatic Hydrocarbons. Cambridge University Press, New York, pp. 11–25.

IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, 2010. Some non-heterocyclic polycyclic aromatic hydrocarbons and some related exposures. IARC Monogr. Eval. Carcinog. Risks Humans 92, 1–853.

Jung, K.H., Patel, M.M., Moors, K., Kinney, P.L., Chillrud, S.N., Whyatt, R., Hoepner, L., Garfinkel, R., Yan, B., Ross, J., Camann, D., Perera, F.P., Miller, R.L., 2010. Effects of heating season on residential indoor and outdoor polycyclic aromatic hydrocarbons, black carbon, and particulate matter in an urban birth cohort. Atmos. Environ. 44, 4545–4552.

Lorenzini, G., Nali, C., 2005. Lo "smog fotochimico" e gli ossidanti atmosferici. In: Lorenzini, G., Nali, C. (Eds.), Le piante e l'inquinamento dell'aria. Springer-Verlag, Italy, pp. 77–80.

Mastral, A.M., Callén, M.S., 2000. A review on polycyclic aromatic hydrocarbon (PAH) emissions from energy generation. Environ. Sci. Technol. 34, 3051–3057.

Mokrzycki, E., Uliasz-Bocheńczyk, A., 2003. Alternative fuels for the cement industry. Appl. Energy 74, 95–100.

Nadal, M., Wargent, J.J., Jones, K.C., Paul, N.D., Schuhmacher, M., Domingo, J.L., 2006. Influence of UV-B radiation and temperature on photodegradation of PAHs: preliminary results. J. Atmos. Chem. 55, 241–252.

Nascimbene, J., Tretiach, M., Corana, F., Lo Schiavo, F., Kodnik, D., Dainese, M., Mannucci, B., 2014. Patterns of traffic polycyclic aromatic hydrocarbon pollution in mountain areas can be revealed by lichen biomonitoring: a case study in the Dolomites (Eastern Italian Alps). Sci. Total Environ. 475, 90–96.

Nisbet, I.C.T., LaGoy, P.K., 1992. Toxic equivalency factors (TEFs) for polycyclic aromatic hydrocarbons (PAHs). Regul. Toxicol. Pharmacol. 16, 290–300.

Odabasi, M., Cetin, E., Sofuoglu, A., 2006. Determination of octanol–air partition coefficients and supercooled liquid vapor pressures of PAHs as a function of temperature: application to gas–particle partitioning in an urban atmosphere. Atmos. Environ. 40, 6615–6625.

Panther, B.C., Hooper, M.A., Tapper, N.J., 1999. A comparison of air particulate matter and associated polycyclic aromatic hydrocarbons in some tropical and temperate urban environments. Atmos. Environ. 33, 4087–4099.

Pellegrini, E., Bertuzzi, S., Candotto Carniel, F., Lorenzini, G., Nali, C., Tretiach, M., 2014. Ozone tolerance in lichens: a possible explanation from biochemical to physiological level using *Flavoparmelia caperata* as test organism. J. Plant Physiol. 171, 1514–1523.

Protano, C., Guidotti, M., Owczarek, M., Fantozzi, L., Blasi, G., Vitali, M., 2014. Polycyclic Aromatic Hydrocarbons and metals in transplanted lichen (*Pseudevernia furfuracea*) at sites adjacent to a solid-waste landfill in central Italy. Arch.

Environ. Contam. Toxicol. 66, 471–481.

Schauer, C., Niessner, R., Ulrich, P., 2003. Polycyclic aromatic hydrocarbons in urban air particulate matter: decadal and seasonal trends, chemical degradation, and sampling artifacts. Environ. Sci. Technol. 37, 2861–2868.

Schrlau, J.E., Geiser, L., Hageman, K.J., Landers, D.H., Massey Simonich, S., 2011. Comparison of lichen, conifer needles, passive air sampling devices, and snowpack as passive sampling media to measure semi-volatile organic compounds in remote atmospheres. Environ. Sci. Technol. 45, 10354–10361.

Shukla, V., Upreti, D.K., 2009. Polycyclic aromatic hydrocarbon (PAH) accumulation in lichen, *Phaeophyscia hispidula* of DehraDun city, Garhwal Himalayas. Environ. Monit. Assess. 149, 1–7.

Shukla, V., Patel, D.K., Upreti, D.K., Yunus, M., 2012. Lichens to distinguish urban from industrial PAHs. Environ. Chem. Lett. 10, 159–164.

Simonich, S.L., Hites, R.A., 1994. Vegetation-atmosphere partitioning of polycyclic aromatic hydrocarbons. Environ. Sci. Technol. 28, 939–943.

Singh, D.P., Gadi, R., Mandal, T.K., Saud, T., Saxena, M., Sharma, S.K., 2013. Emissions estimates of PAH from biomass fuels used in rural sector of Indo-Gangetic plains of India. Atmos. Environ. 68, 120–126.

Tretiach, M., Adamo, P., Bargagli, R., Baruffo, L., Carletti, L., Crisafulli, P., Giordano, S., Modenesi, P., Orlando, S., Pittao, E., 2007. Lichen and moss bags as monitoring devices in urban areas. Part I: influence of exposure on sample vitality. Environ. Pollut. 146, 380–391.

Tretiach, M., Candotto Carniel, F., Loppi, S., Carniel, A., Bortolussi, A., Mazzilis, D., Del Bianco, C., 2011. Lichen transplants as a suitable tool to identify mercury pollution from waste incinerators: a case study from NE Italy. Environ. Monit. Assess. 175, 589–600.

Van der Wat, L., Forbes, P.B.C., 2015. Lichens as biomonitors for organic air pollutants. Trends Anal. Chem. 64, 165–172.

Wild, E., Dent, J., Thomas, O.G., Jones, K.C., 2005. Real-time visualization and quantification of PAH photodegradation on and within plant leaves. Environ. Sci. Technol. 39, 268–273.

Zhu, N., Schramm, K.W., Wang, T., Henkelmann, B., Fu, J., Gao, Y., Wang, Y., Jiang, G., 2015. Lichen, moss and soil in resolving the occurrence of semi-volatile organic compounds on the southeastern Tibetan Plateau, China. Sci. Total Environ. 518–519, 328–336.