Bull Environ Contam Toxicol (2009) 83:852–858
DOI 10.1007/s00128-009-9792-7

Monitoring of Traffic-Related Pollution in a Province of Central Italy with Transplanted Lichen *Pseudovernia furfuracea*

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Received: 21 January 2009 / Accepted: 15 May 2009 / Published online: 29 May 2009

Abstract The ability of transplanted lichen *Pseudevernia furfuracea* to biomonitor specific airborne pollutants (heavy metals and polycyclic aromatic hydrocarbons—PAHs) was investigated at five stations with different traffic densities in Viterbo, Italy. Exposed lichen showed high levels of all analysed pollutants; greatest values were for Zn (147–252 μg/g dw), Pb (24.9–34.6 μg/g dw), fluoranthene (37–107 ng/g dw), pyrene (23–124 ng/g dw). Comparison between contaminants concentration in lichens before and after exposure showed "accumulation" or "severe accumulation" rates in more than 90% of each substance. Besides, *Pseudevernia furfuracea* accumulated airborne PAHs in a manner that was proportional to traffic density.

Keywords Pollution · Lichen · Heavy metals · Polycyclic aromatic hydrocarbons

Vehicular traffic is a primary contributor to urban air pollution. Traffic can cause humans to be exposed to airborne contaminants and suffer health consequences related to this exposure, such as pulmonary dysfunction, cardiovascular dysfunction, and certain types of cancer (Narváez et al. 2008). Consequently, understanding the spatial and temporal trend of traffic-related pollutants is important, particularly in urban areas where motor vehicles are the most important source of such pollutants.

The assessment of air pollution is carried out through "environmental monitoring" that qualitatively and quantitatively measures the presence and distribution of major pollutants at a specific time and location.

Traditionally, airborne contaminants are monitored with automatic machines that first sample them and then analyse their various physical and chemical parameters.

There are substantial costs and difficulties associated with managing the monitoring programs in large urban areas. Therefore, in most cases, traffic pollution is only monitored at a few fixed stations.

Researchers have investigated the possibility of assessing air pollution in alternative ways, such as by using different plant species as bioindicators (Hijano et al. 2005; Suzuki et al. 2008) or as bioaccumulators (Augusto et al. 2007; Blasco et al. 2008; Bermudez et al. 2008). In the first instance, the chosen plant must have a high sensitivity toward specific pollutants and must show clear and quantifiable symptoms; in the second, the plant must be able to tolerate and accumulate pollutants in its tissue.

In particular, lichens function as efficient bioindicating and bioaccumulating organisms, because of several factors. Lichens are perennial, maintain uniform morphology over time, grow slowly, and are dependent on atmospheric deposition for their mineral nutrition. Lichens have no roots and adsorb water and nutrients directly from the air; consequently, they may co-adsorb other substances from the atmosphere, including pollutants.

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Different species of lichen have been used by researchers to monitor certain airborne pollutants, such as heavy metals, nitrogen oxides, ozone, and, more rarely, polycyclic aromatic hydrocarbons (PAHs) (Guidotti et al. 2003; Augusto et al. 2007; Brunialti and Frati 2007; Nali et al. 2007; Baptista et al. 2008; Blasco et al. 2008).

The general objective of this study was to evaluate whether the lichen species *Pseudevernia furfuracea* can be used as biomonitor of specific traffic-related air pollutants. Target analyses included the following: Pb, which is present in petrol and is released after combustion; Cd, Cr, Cu, Ni, Zn, which originate from various vehicle components, such as tyres and brakes; and PAHs, which derive from combustion processes. The specific aim of the study was to evaluate whether different levels of traffic-related air pollution may lead to different levels of bioaccumulation of contaminants in exposed lichen.

Materials and Methods

In February 2005, the epiphytic lichen *P. furfuracea* was collected in a remote area of Mount Terminillo (1,670 m), a mountain in the Rieti province in central Italy.

Lichen was sampled from the bark of beech trees at more than 1 m above ground level. The metals and PAH concentrations (blank) of this sample were tested.

The entire sample was then divided into 10 subsamples of about 10 g each. All subsamples were placed in separate nylon netting bags. We chose five locations, at different traffic intensity levels, in the city of Viterbo, a province in central Italy.

Viterbo is a typical urban area. In 2005, its population was estimated to be 61,067. The city has a mild climate throughout the year, and does not receive much rain. Moreover, the numbers of cars per 1,000 residents in Viterbo is one of the highest in Italy. In 2004, the national rate was 581.1 cars, while the metric in Viterbo was 733.1 cars.

The five locations were classified as high traffic, low traffic, or no traffic areas based on municipal data (Fig. 1).

At each monitoring station, two sample bags were hung, 2 m above ground level, on two trees. After 3 months, all of the samples were retrieved and taken to our laboratory. Each sample was kept at 40°C for 48 h to permit dehydration. Then, extraneous materials such as dust, leaves, and chipping were removed with a microscope. Each sample was then ground with an agate mortar.

A determination of PAH content was conducted using a GC-MS (HP 6890 GC fitted with HP 5973 MS), equipped with a HP5-MS column (25 m × 0.25 mm × 0.25 μm). A total of 2 g of finely chopped lichen was placed into a 40 ml glass vial and 30 ml of cyclohexane was added to the vial. The vial was closed with a PTFE-lined screw cap and extracted for 30 min at room temperature in an ultrasonic bath. The final extract was filtered through filter paper to remove particles. The extraction was repeated with another 30 ml and the combined extracts were concentrated at 2 ml prior to purification. A glass column (1 cm × 25 cm) was prepared in the laboratory by using a slurry of 10 g of activated silica gel in dichloromethane. After the elution of dichloromethane, 2 cm of anhydrous sodium sulphate was added at the top of the silica gel. The column was pre-eluted with 40 ml of pentane. The eluate was discarded and the 2 ml sample extract was transferred onto the column using an additional 2 ml cyclohexane to complete the transfer. About 25 ml of pentane were then added to the column and this eluate was also discarded. Finally, the sample was eluted with 25 ml of a dichloromethane-pentane (2:3 v/v)

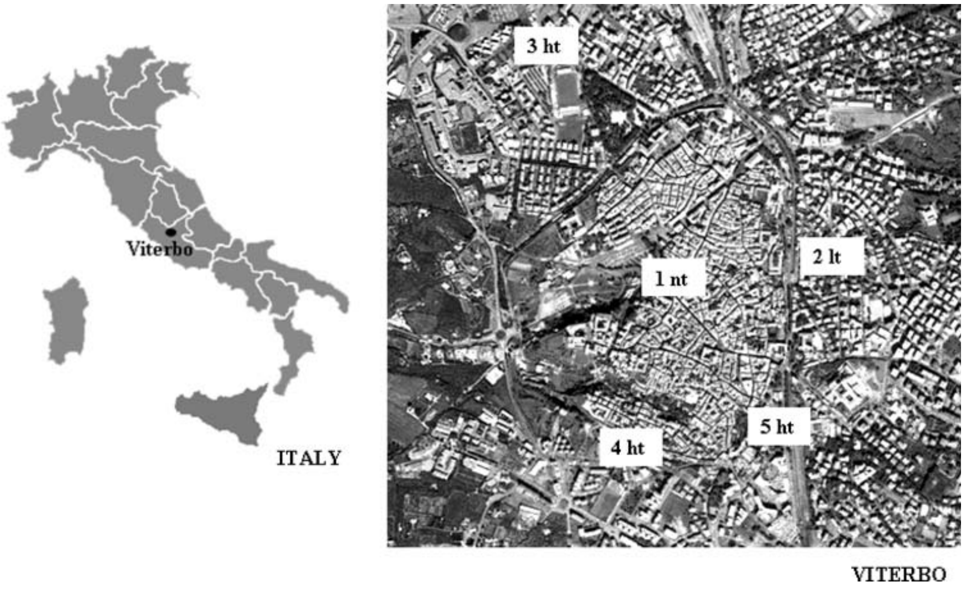

Fig. 1 Location of our study area in Italy and map of the individual sampling sites in Viterbo (ht: high traffic; lt: low traffic; nt: no traffic)

mixture into a 100 ml flask, concentrated to 1 ml by rotavapor. A total of 50 µl of internal standard (perylene d12 at 40 µg/ml in isooctane) was added.

Gas chromatographic conditions were as follows: the split/splitless injector (splitless mode) operated at 300°C, and the purge valve was opened at 1 min. The oven was held at 80°C for 3 min and ramped up to 180°C at 50°C/min. This was held constant for 2 min, before increasing again at 7°C/min to 290°C, where it remained constant for another 10 min. The MS operating conditions were as follows: EM 2,500 eV, electron energy 70 eV, dwell time 50 ms/ion, source temperature 175°C, transfer line 320°C, operating mode: selected ion monitoring (SIM).

The linearity of the method was evaluated in the range 5–500 µg/kg. The calibration curve showed an $r^2 > 0.99$. The repeatability of the method was assessed by analysing six samples of lichen that had previously been extracted with the extraction mixture, supplemented at 100 µg/kg with a standard mixture (EPA PAHs standard mixture at 2,000 µg/ml in methanol - Supelco). The average recovery rate (%), relative standard deviations (RSD) and limits of quantification (LOQ) are reported in Table 1.

A determination of metal content was conducted by graphite furnace atomic absorption spectrometry (AAS Varian Spectra AA 220Z). A total of 150 mg of finely chopped lichen was placed into a “teflon bomb” with 7 ml of 63% HNO_3, 3 ml of 30% H_2O_2 and 0.2 ml of 40% HF. The container was closed with the appropriate teflon screw cap and placed in an oven at 120°C for 3 h. The container was then removed from the oven and cooled at room temperature. The mixture was then transferred into a 50 ml glass flask, and bi-distilled water was then added to the flask. In order to eliminate the matrix effect, quantitative analyses were conducted using the standard addition method. The repeatability of the method was assessed by analysing a reference standard material (BCR No. 482 – heavy metals in lichens) and repeating this procedure six times. The certificated values, recorded values, average recovery, RSD and LOQ for each metal are reported in Table 1.

Results and Discussion

PAHs and metal concentrations for the five selected stations in the *P. furfuracea* lichen samples after 3 months of exposure and in the same lichens before exposure (blank) are reported in Table 2. For each location, the recorded

Table 1 Certificated and recorded values for all metal elements according to BCR No. 482: Heavy metals in lichens, and average recovery rates (%), relative standard deviations (RSDs) and limits of quantification (LOQs) for each compound

Metal elements	Certificated value ± uncertainty (µg/g dw)	Recorded value (µg/g dw)	Recovery (%)	RSD (%)	LOQ (µg/g dw)
Cd	0.56 ± 0.02	0.57	101.8	12.3	0.02
Cr	4.12 ± 0.15	3.92	95.2	11.6	0.03
Cu	7.03 ± 0.19	7.12	101.3	13.2	0.03
Ni	2.47 ± 0.07	2.49	100.8	10.8	0.03
Pb	40.9 ± 1.4	40.8	99.7	6.7	0.01
Zn	100.6 ± 2.2	101.1	100.5	11.0	0.02
PAHs			Recovery (%)	RSD (%)	LOQ (ng/g dw)
Naphthalene (Naph)			75	4.5	1
Phenanthrene (Phen)			72	6.5	1
Anthracene (Anth)			75	3.4	1
Fluoranthene (Flu)			68	3.8	1
Pyrene (Pyr)			75	4.1	1
Benzo(*a*)anthracene (B(a)A)			77	3.0	1
Chrysene (Chyr)			78	4.1	1
Benzo(*b*)fluoranthene (B(b)F)			76	5.1	1
Benzo(*k*)fluoranthene (B(k)F)			83	3.7	1
Benzo(*a*)pyrene (B(a)Py)			77	3.8	1
Indino(*1,2,3cd*)pyrene (IP)			79	4.2	1
Dibenzo(*a,h*)anthracene (D(a,h)A)			73	4.8	1
Benzo(*g,h,i*)perylene (B(ghi)P)			75	4.5	1

Table 2 Mean concentrations of individual PAHs, total PAH concentration (ng/g dw) and heavy metal elements (μg/g dw) found in both unexposed (negative control) and exposed *Pseudovernia furfuracea* lichen at every sampling station

Metal elements	Blank	1 NT	2 LT	3 HT	4 HT	5 HT
Cd	0.5	2.8	6.5	6.2	5.9	7.0
Cr	1.3	4.5	4.3	5.2	5.8	7.1
Cu	5.3	7.9	5.5	13.9	18.8	23.0
Ni	1.4	2.6	2.3	3.5	3.9	4.2
Pb	8.0	26.7	24.9	31.7	29.7	34.6
Zn	36.0	147.0	162.0	153.0	197.0	252.0
PAH	Blank	1 NT	2 LT	3 HT	4 HT	5 HT
Naph	<LOQ[a]	4.0	6.0	6.0	5.0	3.0
Phen	12.0	49.0	41.0	64.0	39.0	25.0
Anth	2.0	38.0	26.0	23.0	17.0	21.0
Flu	14.0	37.0	49.0	107.0	74.0	68.0
Pyr	5.0	23.0	28.0	124.0	62.0	75.0
B(*a*)A	<LOQ[a]	2.0	1.0	9.0	2.0	10.0
Chyr	<LOQ[a]	8.0	10.0	27.0	14.0	19.0
B(*b*)F	<LOQ[a]	3.0	1.0	10.0	3.0	8.0
B(*k*)F	<LOQ[a]	2.0	1.0	7.0	2.0	6.0
B(*a*)Py	<LOQ[a]	2.0	1.0	1.0	1.0	<LOQ[a]
IP	<LOQ[a]	<LOQ[a]	4.0	7.0	<LOQ[a]	9.0
D(*a*,.*h*)A	<LOQ[a]	<LOQ[a]	<LOQ[a]	<LOQ[a]	4.0	<LOQ[a]
B(*ghi*)P	<LOQ[a]	<LOQ[a]	4.0	10.0	<LOQ[a]	9.0
Total PAHs (ΣPAHs)	33.0	168.0	171.0	395.0	226.0	250.0
Total PAH comb (ΣPAH comb)	–	77.0	99.0	302.0	162.0	204.0

[a] Below the Limit Of Quantification of the method

values are expressed as the average of the results obtained from both bags in the same sampling station.

The data presented in Table 2 show, compared with the pre-exposure lichen, an increase in the content of all analysed elements in the transplanted lichen.

In particular, elevated concentrations of metals were identified for Zn (from 147.0 to 252.0 μg/g dw) and Pb (from 24.9 to 34.6 μg/g dw). These elements are commonly found in urban environments. Another study recently conducted in Italy on adsorption of heavy metals with the same lichen, reported similar data. In that study, after 3 months of exposure in urban locations, the most concentrated elements were Zn (24.6 μg/g dw) and Pb (16.7 μg/g dw) (Sorbo et al. 2008).

The elevated levels in our study (between 2 and 8 times higher) are most likely a result of vehicular traffic density in Viterbo, as compared with the geographic area monitored by Sorbo et al. (2008).

The total PAH concentrations ranged from 168.0 to 395.0 ng/g dw. It is difficult to compare these results to similar research because very few studies of lichens as PAH biomonitors have been conducted to date.

A recent study performed on the native *Evernia prunastri* lichen reported a total PAH concentration that ranged 638–5,031 ng/g dw (Blasco et al. 2008).

The significant differences between the PAH concentrations reported by Blasco et al. (2008) and our data may be the result of different techniques of material collection (use of native or transplanted lichens). Furthermore, different lichen species show specific bioaccumulation properties, as demonstrated by Bergamaschi et al. (2007).

Additional reasons for the inconsistent findings may be the characteristics of the monitored areas such as urbanisation, climate conditions, and air pollution levels. The investigation by Blasco et al. (2008) and the present study were performed, respectively, in the central Pyrenees and in Viterbo, Italy. Both locations are characterised by differences in geographic morphology, typical weather conditions and atmospheric contamination.

These assumptions are supported by the results of another study carried out in Italy with transplanted *P. furfuracea* lichen (Guidotti et al. 2003) that reported a concentration range of PAHs that was similar to our results (33–130 ng/g dw).

In our study, the highest concentrations were observed for three-ring (22–52%) and four-ring (42–69%) PAH molecules, whereas concentrations of five- and six-ring chemicals were the lowest (always <6% of the total PAHs).

These results are consistent with other research (Blasco et al. 2008; Shukla and Upreti 2008) and may be associated with the distribution of PAHs in the atmosphere. The lighter PAHs, in fact, are generally present in the gas phase, while higher molecular weight PAHs are usually particulate-bound.

Our results show that lichens can accumulate PAHs of all sizes. They can accumulate substances in the gas phase more easily, but can also accumulate compounds bound to particulate matter, albeit in smaller quantities.

A useful way to describe the characteristics of a lichen specimen in respect of biomonitoring and bioaccumulation involves the evaluation of the exposed-to-control ratio (EC ratio), calculated in terms of the ratio between concentration of a compound in the lichen both after and before its exposure to urban environmental conditions (Frati et al. 2005).

The EC ratio for the analysed elements in each location is shown in Fig. 2. When blank analysis for a specific element resulted <LOQ, the corresponding EC ratio was calculated considering blank equal to LOQ. When the concentration of compound in the lichen after exposure resulted <LOQ, the EC ratio was not calculated.

EC ratio values can be interpreted through the scale defined by Frati et al. (2005), and based on the following criteria: severe loss-based EC = 0.00–0.25; somewhat loss-based EC = 0.25–0.75; normal EC = 0.75–1.25; accumulation EC = 1.25–1.75; severe accumulation EC > 1.75. These criteria have been adopted by other authors (Bergamaschi et al. 2007; Sorbo et al. 2008).

It is remarkable that the EC ratio, calculated for each substance and location, was greater than 1.75 in 90% of metals. Thus, most of the EC ratios fell into the "severe accumulation" category. Only one value (3%) fell into the "normal" category and two (6%) fell into the "accumulation" category. Moreover, although Zn and Pb were the main contaminants recorded after 3 months of exposure, the highest accumulation was for Cd. This indicates that

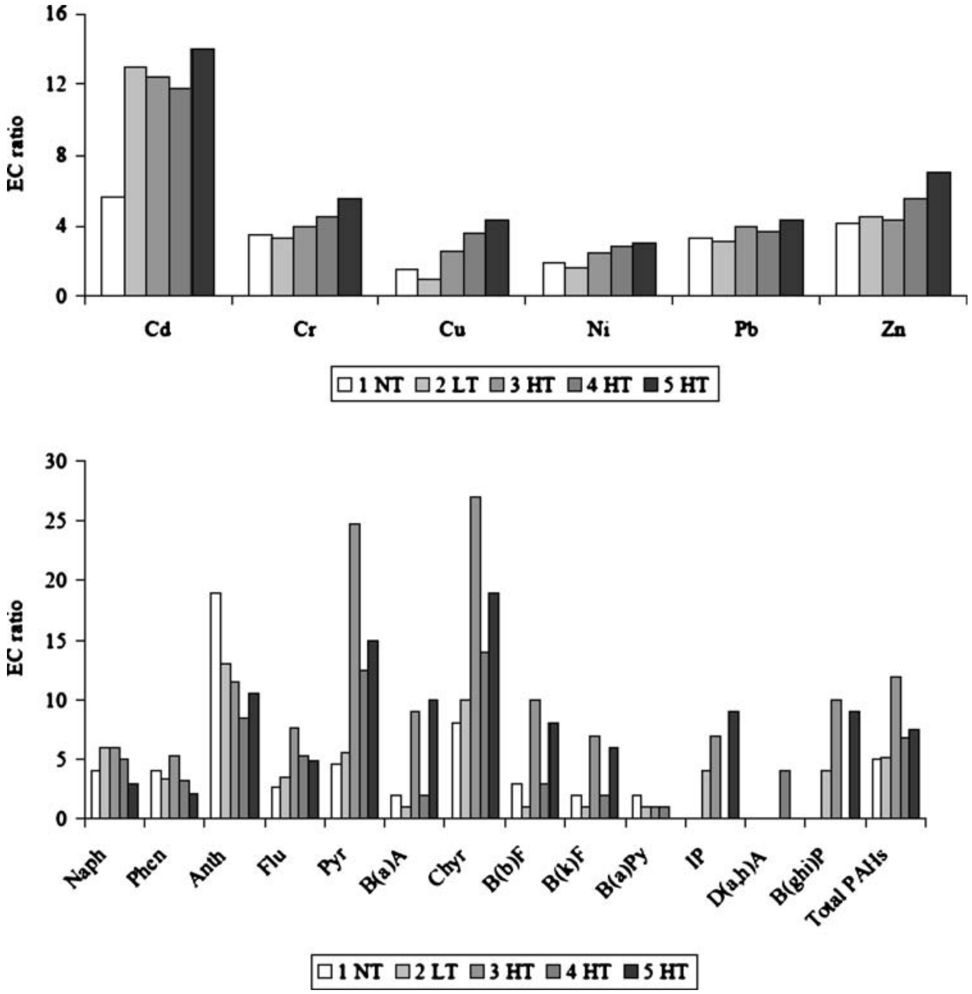

Fig. 2 Exposed-to-control ratio (EC ratio) for all metal elements, individual PAHs, total PAHs at station 1 (no traffic, NT), at station 2 (light traffic, LT), and at stations 3, 4, and 5 (high traffic, HT)

the blank was also exposed to trace metals in the native area. In particular, Zn was the highest metal amount in the blank; this element is widespread in terrigenous sediment and, consequently, may have been absorbed along with soil particles by the native lichen.

Regarding PAHs, the EC ratio was indicative of "severe accumulation" for 91% of the individual PAHs. The remaining 9% of values fell into the "accumulation" category.

The concentration of metals was related to road traffic levels, although the correlation was not strong. Values of all compounds, in fact, were generally higher at stations 3, 4, and 5 – namely the locations selected as high traffic intensity sites. In contrast, it is not possible to identify any differences between the concentrations of metals at stations 1 and 2, which were considered no traffic and light traffic areas, respectively.

Another important concern regarding Pb concentration and its relationship with road traffic is as follows: in Italy, unleaded petrol and catalytic mufflers were introduced in 1992. However, many authors still identified Pb contamination in heavy traffic areas (Monaci et al. 2000; Frati et al. 2005; Sorbo et al. 2008) because of the long residence time of Pb in urban soils and active dust re-suspension.

The concentration of total PAHs is more strongly associated with traffic density than are metal concentrations. As shown in Table 2, the greatest increase in total PAH readings were recorded at stations 3, 4, 5, The smallest total PAH concentration was found at station 1; this area (Fig. 1) is in the centre of Viterbo, but is a restricted traffic district and consequently sees very few vehicles.

Additional confirmation is given by the different concentrations of combustion PAHs (PAHs$_{\text{comb}}$) at the selected stations (Table 2). PAHs$_{\text{comb}}$ reflect PAHs with more than four aromatic rings; they are typically produced during high-temperature combustion processes and consequently are strongly characteristic of vehicle exhaust emissions.

The PAHs$_{\text{comb}}$ concentrations were lowest in areas of no traffic, moderate in areas of light traffic, and highest in areas of dense traffic. At station 1, the percentage was 46%. At station 2, the percentage was 58%. At stations 3, 4, and 5, the percentage was greater than 72%. These results confirm the significant influence of road traffic on the bioaccumulation of pollutants in lichen.

In addition, considering singular PAHs and the relative EC ratio, the accumulation grades were not always linearly related to traffic intensity. This is particularly true for naphthalene, fluoranthene, and anthracene. By contrast, the EC ratio of typical particle-associated PAH was consistently elevated in areas associated with high vehicular emissions, suggesting that different traffic densities principally influence the absorption of PAHs that are bound to particulate matter. Thus, during the three-month exposure period, we conclude that the lichen species *P. furfuracea* absorbed airborne contaminants and accumulated them at rates that were generally proportional to local traffic density.

In particular, the results highlight the ability of this particular lichen species to absorb airborne PAHs and accumulate them in a manner that is somewhat proportional to traffic density.

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