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# Lichens as polycyclic aromatic hydrocarbon bioaccumulators used in atmospheric pollution studies

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**Abstract**

The aim of this work has been to determine the possibility of using lichens as polycyclic aromatic hydrocarbon (PAH) bioaccumulators for the evaluation of atmospheric pollution in the city of Rieti. A lichen sample collected in a remote unpolluted area was divided into 18 sub-samples. These were collocated in nine different stations in the city of Rieti and every 5 months the PAH concentrations were determined. Only phenanthrene, anthracene, fluoranthene, pyrene and chrysene were found at concentrations over the limit of quantification. In the meantime a continuous increase of their concentrations was observed.


*Keywords:* Air analysis; Environmental analysis; Polynuclear aromatic hydrocarbons

## 1. Introduction

Air is normally monitored using special automatic machines that sample and analyse it. Consequently the cost and the difficulty in managing the monitoring of large urban areas appear immediately clear.

In the last few years various scientific investigations have been performed on such kinds of pollution using mosses and lichens as bioindicators [1–4] or as bioaccumulators [5–11]. In the first instance the chosen plant has a high sensitivity towards specific pollutants showing clear and quantifiable symptoms, in the second instance it must be able to tolerate and to accumulate the pollutants in its tissue.

The greater part of the studies on the accumulation of air pollutants by lichens focused on pollution caused by heavy metals. For this reason it was of interest to know if lichens were able to accumulate other pollutants. In a previous work we observed not only a considerable increase in polycyclic aromatic hydrocarbon (PAH) concentrations in lichens growing in the city of Rieti but also a direct correlation with vehicular traffic [12].

PAHs are widespread environmental pollutants. They are formed during incomplete combustion and pyrolysis of organic materials, from both natural and anthropogenic sources, the latter being by far the greatest contributors [13]. The name ''polycyclic aromatic hydrocarbons'' commonly refers to a large

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class of organic compounds containing two or more fused aromatic rings.

The experimental carcinogenicity and mutagenicity shown by many PAHs have resulted in a strong suspicion that they are also human carcinogens [14].

## 2. Experimental

### 2.1. Reagents

A mixture of the 16 US Environmental Protection Agency (EPA) priority PAHs at 100 μg/ml in methylene chloride from Agilent Technologies was used to make up the working standard solutions in hexane. A solution of [$^{2}H_{12}$]perylene at 2 mg/ml in methylene chloride from Supelco was used as internal standard. Hexane, methylene chloride and cyclohexane for pesticide residues were from Carlo Erba. Silica gel 100–200 mesh from Merck was activated for 16 h at 130 °C in a shallow glass tray and then stored in a sealed glass jar inside a desiccator.

### 2.2. Instrumentation

An ultrasonic bath from Branson was used to extract the lichen samples. The sample extracts were filtered with filter paper No. 40 from Whatman. The gas chromatographic–mass spectrometric (GC–MS) analysis were carried out with a Hewlett-Packard (HP) 5971 quadrupole mass spectrometer interfaced with a HP 5890 Series II gas chromatograph equipped with a HP5-MS fused-silica capillary column from HP (30 m×0.25 mm I.D., 0.25 μm phase film). The carrier gas was helium at 1 ml/min at 80 °C.

The GC system was equipped with a split/splitless injector operating in the splitless mode with the purge valve open at 1.0 min. The GC column was held at 80 °C for 3 min and ramped to 180 °C at 50 °C/min, held for 2 min at 180 °C ramped to 290 °C and held at 290 °C for 10 min. The MS operating conditions were: EM 2800 eV; electron energy 70 eV; dwell time 50 ms/ion; source temperature 175 °C; injector temperature 300 °C; transferline temperature 320 °C; operating mode: selected ion monitoring (SIM) with two ions for each PAH as reported in Table 1. The first ion was used for the quantification and the second one was used for the qualitative analysis.

### 2.3. Sampling and sample preparation

In November 1999 the epiphytic lichen *Pseudevernia furfuracea* was collected in a remote area of the Terminillo mountain (1670 m) from beech trees bark at more than 1 m from the ground. This sample was tested to evaluate the PAH concentrations (blank). The entire sample was then divided into 18 subsamples of 30 g and each one was closed in specific bag prepared with nylon net. Eight locations were chosen with different traffic intensity levels (Table 2). For each monitoring station two sample bags were hung on two trees at 2 m from the ground.

Every 5 months about 5 g of lichen was taken from each sample bag and kept in the laboratory for the determination of PAHs. In the laboratory the lichen samples were kept at 40 °C for 48 h in a stove

Table 1
Monitored ions

| PAH | *m/z* | | PAH | *m/z* | |
|---|---|---|---|---|---|
| | First ion | Second ion | | First ion | Second ion |
| Naphthalene | 128 | 129 | Chrysene | 228 | 229 |
| Acenaphthylene | 152 | 153 | Benzo[*b*]fluoranthene | 252 | 126 |
| Acenaphthene | 153 | 154 | Benzo[*k*]fluoranthene | 252 | 126 |
| Fluorene | 166 | 165 | Benzo[*a*]pyrene | 252 | 126 |
| Phenanthrene | 178 | 179 | Indeno[1,2,3-*cd*]pyrene | 276 | 277 |
| Anthracene | 178 | 179 | Dibenzo[*a,h*]anthracene | 278 | 276 |
| Fluoranthene | 202 | 201 | Benzo[*ghi*]perylene | 276 | 277 |
| Pyrene | 202 | 201 | [$^{2}H_{12}$]Perylene | 264 | 260 |
| Benzo[*a*]anthracene | 228 | 229 | | | |

Table 2
Sampling stations

| | |
|---|---|
| Station 1 | Medium traffic intensity—residential area. |
| Station 2 | High traffic intensity—center near a traffic light. |
| Station 3 | Scarce traffic intensity—suburb |
| Station 4 | Medium traffic intensity—in the center near a park. |
| Station 5 | Low traffic intensity—agricultural area 2 km from the city. |
| Station 6 | Low traffic intensity—in the middle of a park. |
| Station 7 | High traffic intensity—in the center near by a level crossing. |
| Station 8 | Medium traffic intensity—suburb |

to permit desorption. The sample was cleaned, removing the extraneous material like dust, leafage and pebbles by the use of a microscope and then it was ground in an agate mortar. A 2-g amount of finely chopped lichen was finally placed with 30 ml of cyclohexane in a 40-ml glass vial with a PTFE lined screw cap and extracted for 30 min at room temperature in an ultrasonic bath. The final extract was filtered through a filter paper to remove particles. The extraction was repeated with another 30 ml of solvent and the combined extract was concentrated to 2 ml before purification.

### 2.4. Purification

The sample extract was purified using EPA method 3630B. A glass chromatography column was prepared in the laboratory by preparing a slurry of 10 g of activated silica gel in dichloromethane. After the elution of the methylene chloride 2 cm of anhydrous sodium sulfate was added to the top of the silica gel. The column was pre-eluted with 40 ml of pentane at about a flux of 2 ml/min. The elute was discarded and, just prior to exposure the sodium sulfate layer to the air, the 2 ml cyclohexane sample extract was transferred onto the column using an additional 2 ml cyclohexane to complete the transfer. Just prior to exposure the sodium sulfate layer to the air, 25 ml of pentane was added to continue the elution of the column. The pentane elute was discarded. The sample was eluted with 25 ml of methylene chloride–pentane (2:3, v/v) mixture into a 100-ml flask and concentrated to 1 ml .

### 2.5. GC–MS analysis

The determination of the analytes was carried out using GC–MS and the quantification was performed with the use of [$^2H_{12}$]perylene as internal standard. The working standard was prepared by a dilution of the EPA PAH mixture from 100 to 0.4 μg/ml in hexane.

### 2.6. Method performance

The linearity of the method was evaluated in the range 5–500 μg/kg. The calibrations curves showed an $r^2$ higher than 0.99.

The repeatability of the method was assessed by analysing five samples of lichens previously extracted with cyclohexane and spiked at a concentration of 100 μg/kg for each component with the EPA PAH mixture. The average recoveries (%), the relative standard deviations (RSDs) and the limits of quantification (LOQs) are reported in Table 3.

## 3. Results and discussion

Fig. 1 shows a chromatogram related to the standard mixture at 0.2 μg/ml. The resolution calculated for the PAH isomeric pairs were: phenanthrene/anthracene 0.55, benzo[*a*]anthracene/chrysene 0.47, benzo[*b*]fluorantene/benzo[*k*]fluorantene 0.21 and indeno[1,2,3-*cd*]pyrene/dibenzo-[*a*,*h*])anthracene 0.41.

Fig. 2 shows the extracted ion chromatogram relative to phenanthrene (ion *m*/*z* 178) anthracene (ion *m*/*z* 178), fluoranthene (ion *m*/*z* 202) and pyrene (ion *m*/*z* 202) for a lichen sample.

Fig. 3 shows the extracted ion chromatogram of the same PAHs for the standard mixture at 0.2 μg/ml.

In Table 4 the sums of PAH concentrations for the

Table 3
Average recoveries (%), relative standard deviations (RSDs) and limits of quantification (LOQs)

| PAH | Recovery (%) | RSD (%) | LOQ (μg/kg) | PAH | Recovery (%) | RSD (%) | LOQ (μg/kg) |
|---|---|---|---|---|---|---|---|
| Acenaphthylene | 43 | 12.0 | 0.6 | Benzo[*a*]anthracene | 72 | 3.4 | 1.8 |
| Acenaphthene | 48 | 13.0 | 0.5 | Chrysene | 75 | 2.7 | 1.8 |
| Fluorene | 65 | 13.0 | 0.6 | Benzo[*b*]fluoranthene | 76 | 3.6 | 2.0 |
| Phenanthrene | 73 | 3.4 | 1.2 | Benzo[*k*]fluoranthene | 73 | 4.3 | 2.0 |
| Anthracene | 71 | 5.9 | 1.2 | Benzo[*a*]pyrene | 86 | 3.3 | 2.4 |
| Fluoranthene | 73 | 2.9 | 1.2 | Indeno[1,2,3-*cd*]pyrene | 74 | 9.1 | 3.6 |
| Pyrene | 67 | 3.3 | 1.2 | Dibenzo[*a,h*]anthracene | 74 | 2.9 | 3.6 |

whole investigated period are reported. In this computation only PAHs present at concentrations higher than the LOQ were considered.

For each station the reported value is the average of the results obtained for the two sample bags located in the sampling station.

As we can see the higher increases in PAH concentrations are present at stations 6 and 7 exhibiting a high traffic intensity. For all the stations the greater increases were observed in the period between September 2000 and February 2001, probably due to the presence in the atmosphere of PAHs produced from the domestic heating systems (which in Italy are often fed with naphtha and coke). In Italy, the working period of the domestic heating usually lasts from November 15 to March 31.

The only PAHs present at concentrations over the LOQs were: phenanthrene, anthracene, fluoranthene, pyrene and chrysene. Their concentrations in the samples of February 2001 are reported in Table 5. These PAHs (three or four rings in their structure) are the same found by Wenzel et al. [15] in pine needles and for them the authors assess a high bioconcentration. At the same time these PAHs are those more present in vehicle emissions [16] and are partially or totally present in the vapour phase of the atmosphere [17].

Other PAHs (five or more rings in their structure) present in the atmosphere in high concentrations not present in lichens may be because they were almost exclusively adsorbed on suspended particulate matter [17].

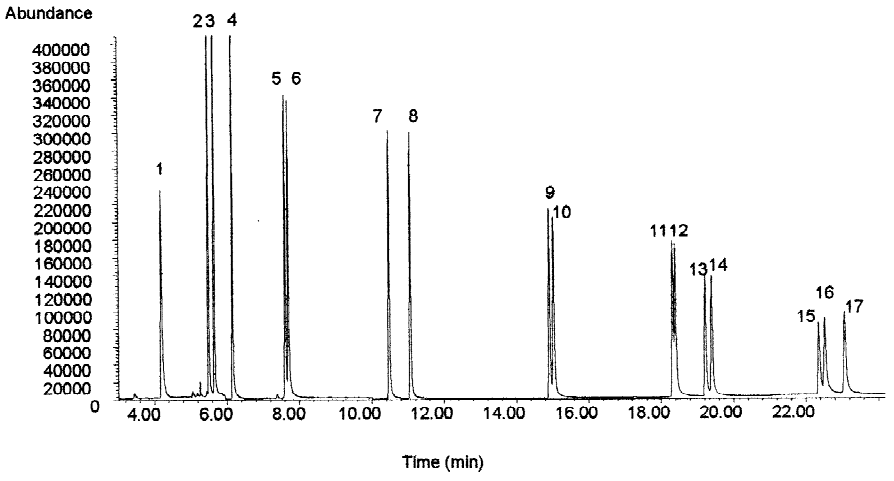


Fig. 1. Total chromatogram related to the standard mixture at 0.2 μg/ml: (1) naphthalene, (2) acenaphthylene, (3) acenaphthene, (4) fluorene (5) phenanthrene, (6) anthracene, (7) fluoranthene, (8) pyrene, (9) chrysene, (10) chrysene, (11) benzo[*b*]fluoranthene, (12) benzo[*k*]fluoranthene, (13) benzo[*a*]pyrene, (14) [$^{2}H_{12}$]perylene, (15) indeno[1,2,3-*cd*]pyrene, (16) dibenzo[*a,h*]pyrene, (17) benzo[*ghi*]perylene.

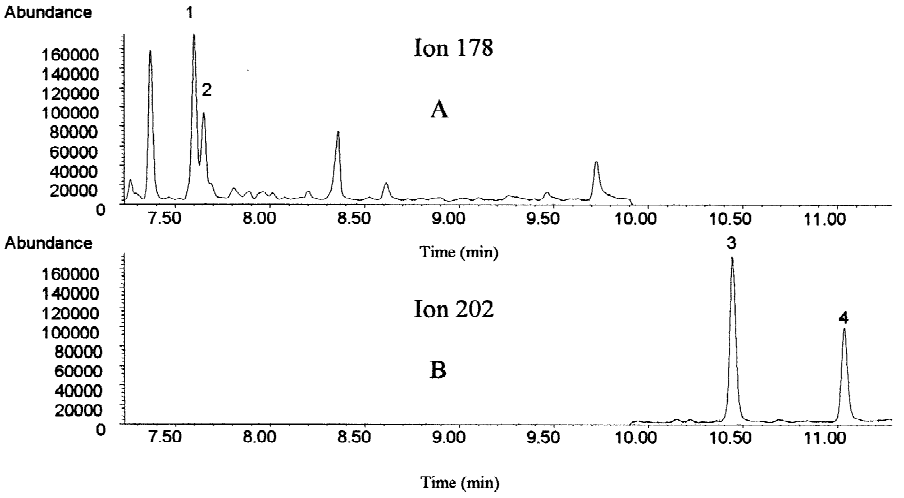


Fig. 2. Extracted ion chromatograms obtained from a lichen sample extract. Chromatogram A (ion $m/z$ 178): phenanthrene (1) and anthracene (2). Chromatogram B (ion $m/z$ 202): fluoranthene (3) and pyrene (4).

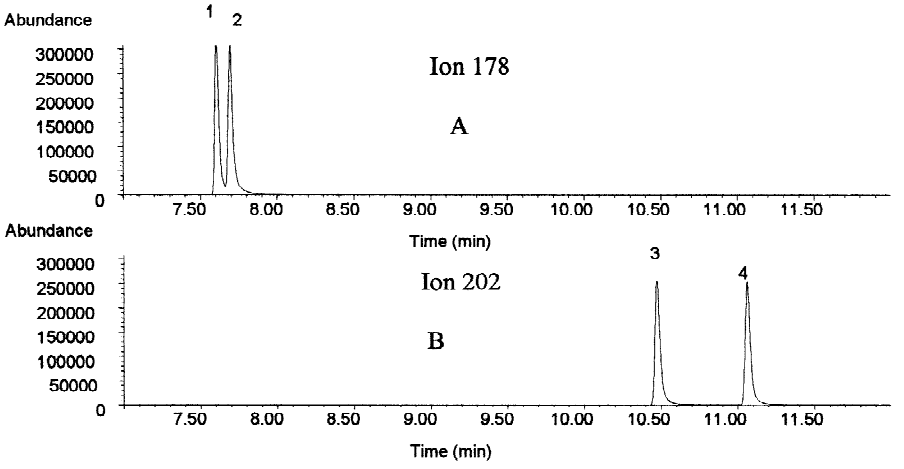


Fig. 3. Extracted ion chromatograms obtained from the standard mixture at 0.2 μg/ml. Chromatogram A: phenanthrene (1) and anthracene (2) (ion $m/z$ 178). Chromatogram B: fluoranthene (3) and pyrene (4) (ion $m/z$ 202).

Table 4
Total PAH (μg/kg) concentrations

| | Station No. | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| November 1999 | 36 | 36 | 36 | 36 | 36 | 36 | 36 | 36 |
| April 2000 | 62 | 125 | 50 | 67 | 33 | 61 | 130 | 84 |
| September 2001 | 68 | 150 | 66 | 71 | 50 | 61 | 154 | 94 |
| February 2001 | 151 | 368 | 98 | 114 | 110 | 98 | 322 | 114 |
| July 2001 | 163 | 223 | 101 | 120 | 103 | 106 | 375 | 141 |

Table 5
Concentrations of phenanthrene, anthracene, fluoranthene, pyrene, chrysene and sum of PAHs (μg/kg) in the samples of February 2001

| | Station No. | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| Phenanthrene | 19 | 44 | 26 | 28 | 17 | 20 | 58 | 25 |
| Anthracene | 14 | 27 | 10 | 11 | 10 | 13 | 28 | 13 |
| Fluoranthene | 20 | 45 | 19 | 22 | 14 | 28 | 39 | 25 |
| Pyrene | 15 | 34 | 12 | 12 | 11 | 20 | 25 | 16 |
| Chrysene | 4 | 8 | 2 | 3 | 4 | 5 | 9 | 3 |
| PAHs | 72 | 158 | 69 | 76 | 56 | 86 | 159 | 82 |

## 4. Conclusions

This study confirms the important role of the domestic heating and vehicle traffic on the atmospheric PAH concentrations. Moreover seems that phenanthrene, anthracene, fluoranthene and pyrene, probably because partially or totally in the vapour phase in the atmosphere, can be more easily bioaccumulated in lichens. So, the present work, while not providing an efficient system in the interpretation of transport mechanisms through the cell membranes in lichens, but an understanding of which can complete this investigation.

In conclusion the use of lichens can be proposed as an inexpensive and more available natural method to assess and continuously monitor urban atmospheric pollution caused mostly by combustion processes such as domestic heating and vehicle traffic.

## References

[1] F.D.H. Macdowall, E.I. Maccumal, Can. J. Plant Sci. 44 (1964) 410.
[2] D.J. Ball, London Naturalist 60 (1981) 27.
[3] P.L. Nimis, M. Castello, M. Perotti, Lichennologist 22 (1990) 333.
[4] H. Zechmeister, Environ. Pollut. 73 (1995) 89.
[5] A. Angoletta, A.M. Bentivoglio, D. Giusto, Inquinamento 35 (1993) 56.
[6] G. Eriksson, S. Jensen, H. Kylin, W. Strachan, Nature 341 (1995) 367.
[7] G. Huhn, H. Schultz, H.J. Stark, Water Air Soil Pollut. 84 (1995) 367.
[8] K.J. Puckett, Can. J. Bot. 54 (1976) 2695.
[9] S. Loppi, L. Nelli, S. Ancora, R. Barbagli, Environ. Monitor. Assess. 45 (1997) 81.
[10] R. Ferrara, B.E. Parerti, R. Greader, Water Air Soil Pollut. 56 (1991) 219.
[11] R. Bargali, Sci. Total Environ. 176 (1995) 121.
[12] M. Owczarek, M. Guidotti, G. Blasi, C. De Simone, A. De Marco, Fresenius Environ. Bull. 42 (2001) 10/1.
[13] M.L. Lee, M. Novotny, K.D. Bartle, Analytical Chemistry of Polycyclic Aromatic Compounds, Academic Press, New York, 1981.
[14] IARC, in: Polynuclear Aromatic Compounds, Part 1, Chemical, Environmental and Experimental Data, IARC Monograph on the Evaluation of the Carcinogenic Risk of Chemicals To Humans, Vol. 32, International Agency for Research on Cancer (IARC), Lyon, 1983.
[15] K.D. Wenzel, L. Weisflog, M. Manz, A. Hubert, G. Schuurmann, Fresenius Environ. Bull. 47 (2000) 9.
[16] G. Salvi, A. Cosalini, Riv. Comb. 38 (1984) 159.
[17] R.W. Coutan, L. Brown, J.C. Chuang, R.M. Riggin, R.G. Lewis, Atmos. Environ. 22 (1988) 403.