

Correlation between the nitrogen concentration of two epiphytic lichens and the traffic density in an urban area

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“Capsule”: *Nitrogen concentrations of the lichen *Physcia adscendens* are related to traffic exposure.*

Abstract

A field experiment was carried out in the urban environment of the Grenoble area using two epiphytic lichens: the nitrophytic *Physcia adscendens* and the acidiphytic *Hypogymnia physodes*. Two complementary studies characterized this experiment. Firstly, a sampling of the two lichens in 48 sites randomly located throughout the Grenoble area indicated that roads (size and proximity to sampling sites) influenced the nitrogen concentrations of *P. adscendens*, but not those of *H. physodes*. Secondly, to study more accurately the influence of roads, a traffic index was calculated and applied along two transects located perpendicularly to urban motorways. Significant positive correlations were found between this traffic index and the total nitrogen concentration of *P. adscendens*.

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1. Introduction

Lichens have been shown to be outstanding bioindicators in a wide range of air pollution studies. This is due to the physiological properties of the lichen thallus, namely its slow growth and its effectiveness in absorbing soluble and insoluble mineral nutrients from ambient air and rain with little subsequent loss (Ahmadjian and Hale, 1973; Ferry et al., 1973; Nash, 1996). Because of their structure, lichens depend mainly on atmospheric deposition for their nutrition, especially for their nitrogen supply.

The different forms of nitrogen can be supplied by two major sources: mainly ammonia in rural environments and mainly nitrogen oxides in urban environments. According to circumstances, the major inputs of atmospheric nitrogen occur as dry deposition of gaseous nitric acid (HNO₃), ammonia (NH₃) and nitrogen dioxide (NO₂) and wet deposition of nitrate (NO₃⁻) and ammo-

niun (NH₄⁺) (Fowler et al., 1991; Hargreaves et al., 1992; Pitcairn et al., 1995).

In rural areas, the main effects of nitrogen deposition on lichens can result in changes of the communities (Loppi and De Dominicis, 1996; Van Dobben and De Bakker, 1996), a greater occurrence of nitrophytic species (De Bakker, 1989; Ruoss et al., 1992; Kirschbaum and Hanewald, 2000), often associated with a rise of bark pH (De Bakker, 1989; Van Herk, 1993; Van Dobben and De Bakker, 1996; Van Herk, 2001), a decrease in the biodiversity (Cepeda and Garcia Rowe, 1998; Van Dobben and Ter Braak, 1998), and an increasing uptake of nitrogen near sources of pollution (Søchting, 1995; Ruoss, 1999). Positive relationships have also been found between the nitrogen content of some bryophytes and nitrogen deposition (Pitcairn et al., 1995) and NO_x and NH₃ deposition (Poikolainen et al., 1998).

In urban areas or close to roads and motorways, studies have shown the positive correlation between traffic density and levels of different primary pollutants (Colwill, 1975; Kuhler et al., 1994; Ward, 1990). High levels of pollutants on verges can cause damage to higher plants (Flückiger et al., 1978; Thompson et al.,

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1984; Kammerbauer et al., 1986; Spencer and Port, 1988; Spencer et al., 1988; Sharma, 1987; Sauter and Pambor, 1989), and greater bioaccumulation of nitrogen (Port and Thompson, 1980). Pearson et al. (2000) showed a very good correlation between traffic exposure and tissue ^{15}N ($^{15}\text{N}/^{14}\text{N}$) in different mosses. However, few studies dealing with lichens have been carried out. For the past decade, the changes in the lichen communities have been observed in the Rhône-Alpes area (south-east region of France), with an increase of the nitrophytic lichens in urban areas (Gombert, 1999; Khalil and Asta, 1998; Khalil, 2000) and in some French Alps valleys close to motorways (Paul, 2000).

One of our hypotheses was that tissue nitrogen content of lichens collected near roadsides would relate to traffic or exposure. The aim of this experiment was to study the influence of urban roads on the nitrogen concentration of a nitrophytic lichen, *Physcia adscendens* and an acidiphytic one, *Hypogymnia physodes* in the Grenoble area (France). Lichen nitrogen concentrations were analyzed and mapped for the whole urban area (48 sites) and along two transects located perpendicularly to urban motorways. A traffic index based on traffic density and road vicinity was calculated to characterize each lichen sampling site of the two transects.

2. Material and methods

2.1. Study area and transects

The Grenoble area (Isère, France, Fig. 1) is situated in the Northern French Alps in south-east France where the climate is characterized by an average annual rainfall of 1000 mm and an average annual temperature of 12 °C. It is located at the confluence of two rivers (Isère and Drac), in a Y-shaped alluvial plain at an altitude of 200–330 m. The Grenoble area forms an urban agglomeration with a total population of about 375,000 inhabitants.

Two transects were carried out within this area. Transect 1, located in the north-east of the study area (city of Meylan) (Fig. 1), at an altitude 220–330 m, was about 2000 m long. This transect was situated perpendicularly to the *A 41 Motorway (MW)* and crossed three other secondary roads characterized by less traffic: *Vercors Avenue (VA)*, *Main Road (MR)* and *Chartreuse Avenue (CA)*. Transect 2, located in the south-east area of the studied area (city of Saint Martin d'Hères) (Fig. 1), at a consistent altitude of 220 m, was about 1000 m long. This transect was also situated perpendicularly to a motorway (*South Motorway (SW)*) and crossed two other secondary roads: *Wallon Avenue (WA)* in the north and *Prevert Avenue (PA)* in the south. No pollution data were available for these two transects.

2.2. Biological materials

Two lichens were used in this study: *Physcia adscendens* (F.) Oliv. and *Hypogymnia physodes* (L.) Nyl. *P. adscendens* was chosen because of its great frequency in the Grenoble area, and because it is increasingly found in monospecific lichen communities in urban areas (Gombert, 1999; Khalil, 2000). This nitrophytic lichen prefers to grow on deciduous tree bark enriched with dust or nutrients. *H. physodes*, which prefers acidic barks, has been used in numerous bioindication studies dealing with nitrogen (Bruteig, 1993; Metzger, 1993; Søchting, 1995; Poikolainen et al., 1998). These two lichens possess green algae as their photosymbiont, but not cyanobacteria which would allow them to assimilate atmospheric N_2 .

2.3. Sampling

Nitrogen concentrations in the two lichens were determined at 48 stations randomly located throughout the whole Grenoble area with samples of *P. adscendens* and *H. physodes* collected at 44 and 34 of these stations, respectively. Lichen nitrogen concentrations were also analyzed on two transects. In transect 1, samples of *P. adscendens* and *H. physodes* were collected at 13 and 7 stations, respectively, and for transect 2, 13 and 4 stations respectively.

Several tree species (poplar, maple, ash and walnut) were present in each sampling site. In order to obtain representative sampling and enough lichen biomass, 20 thalli were sampled on several neighbouring trees in each site. Lichen thalli were collected with a knife above 1 m to about 2 m on the trunks to minimize the influence of animal wastes and chemical spraying. Lichens were sampled in no specific orientation around the trees according to the frequency of the species. Sampling was carried out during two dry periods (to collect dry thalli and to avoid leaching): in July 1998 (for both the mapping and the transect studies) and in November 1998 (for the transect study only).

2.4. Lichen treatment and nitrogen analysis

For each lichen sample, all unwashed thalli were finely ground with a steel ball grinder. Samples were dried for 24 h at 105 °C. Total lichen nitrogen was analyzed in triplicate by gaseous chromatography and expressed as a percentage of dry weight. To evaluate the possible influence of washing on the nitrogen content, samples collected on transect 1 in November were also washed (four rapid immersions into distilled water) and analyzed according to the same procedure.

2.5. Traffic index

Under the assumption that roads can be considered as sources of gaseous effluents, which are submitted to

diffusion processes, a one-dimensional simplified model with a continuous source term was proposed. The form of the solution of this model was given by:

$$C(x) = A \cdot \exp(-B \cdot x^2)$$

where: A is a constant depending on the source rate; B is a constant proportional to the Diffusion Coefficient (fixed for a given medium); x is the distance from the source.

Considering n to represent n several sources, located at x_i abscissa ($i = 1$ to n), the concentration on a given

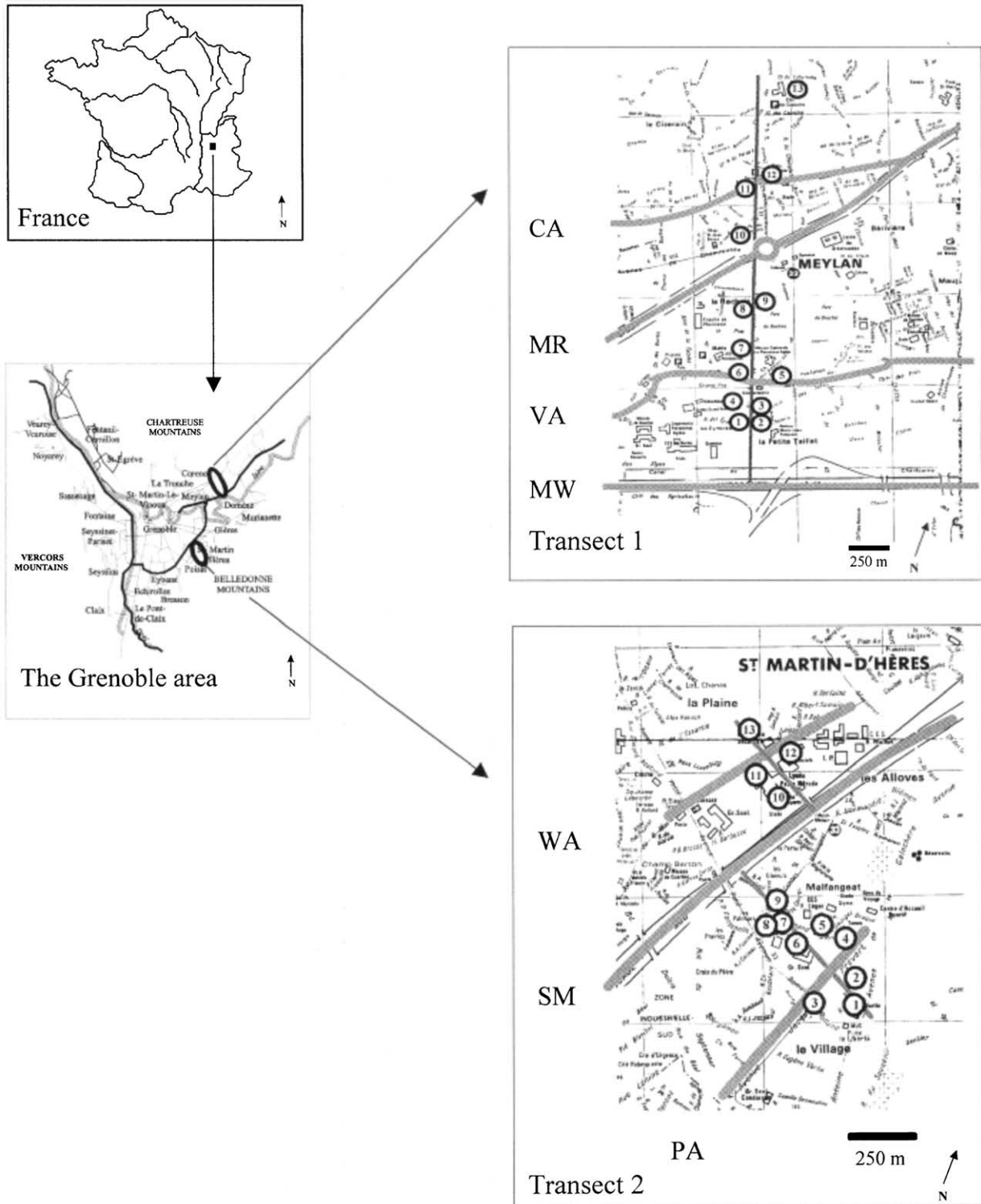


Fig. 1. The study area of Grenoble (France) and the two transects: transect 1 located in the city of Meylan and transect 2 located in the city of Saint Martin d'Hères.

abscissa is the sum of the contributions of the different sources:

$$C(x) = A_1 \cdot \exp(-B \cdot (x - x_1)^2) + \dots + A_i \cdot \exp(-B \cdot (x - x_i)^2) + \dots + A_n \cdot \exp(-B \cdot (x - x_n)^2).$$

In our case, the constant A_i is related to traffic density ($A_i = T_i$). The constant B ($B = K$), which can be estimated with the decreasing concentration of any gaseous effluent with the distance from a single source, is fitted to our data.

$$\begin{aligned} \text{Traffic index} &= \sum_{i=1}^n T_i \cdot \exp(-K(x - x_i)^2) \\ &= \sum_{i=1}^n T_i \cdot \exp(-K \cdot d_i^2) \end{aligned}$$

where: T_i values are for transect 1: $T_{\text{Motorway}} = T_{\text{MW}} = 10$; $T_{\text{Main Road}} = T_{\text{MR}} = 3$; $T_{\text{Vercors Avenue}} = T_{\text{VA}} = 1.5$; $T_{\text{Chartreuse Avenue}} = T_{\text{CA}} = 1.5$, and for transect 2: $T_{\text{South Motorway}} = T_{\text{SM}} = 10$; $T_{\text{Prevert Avenue}} = T_{\text{PA}} = 1.5$; $T_{\text{Wallon Avenue}} = T_{\text{WA}} = 1.5$; K is $4 \cdot 10^{-5}$; d_i is the distance (m) from the source, i.e. the distance from each road.

2.6. Statistical tests

For the mapping study, more than 30 sampling sites ($n=44$ for *P. adscendens* samples) were plotted and parametric statistics were applied to the data. The student *t*-test was used to test the differences between the two lichens. The influence of roads on lichen nitrogen concentrations was tested for statistical significance using a single factor one-way analysis of variance (ANOVA). The mapping of lichen nitrogen concentrations was realized by the interpolation method of triangulation (Mapinfo software) by the ASCOPARG (Association pour le Contrôle et la Préservation de l’Air dans la région Grenobloise, Air Monitoring and protection Association in the Grenoble Area).

Due to the low numbers of sampling stations of the two transects ($n=13$), non-parametric statistics were suitable for these data. Therefore, the Spearman’s rank correlation was used to test the relationships between lichen nitrogen concentration and traffic index. The Mann–Whitney test was employed to test the differences between nitrogen concentrations of samples collected on the two transects during different periods. The unilateral Wilcoxon paired-samples test was employed to test the difference between paired washed and unwashed *P. adscendens* samples (transect 1, November).

Significant results are indicated by means of stars: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

3. Results

3.1. Mapping study of lichen nitrogen concentrations

The nitrogen concentrations in lichens obtained in the Grenoble area vary from 1.21 to 3.75% with a mean value of $2.41 \pm 0.70\%$ for *H. physodes* and from 1.75 to 4.21% with a mean value of $2.97 \pm 0.65\%$ for *P. adscendens* (Table 1). These nitrogen concentrations

Table 1
Nitrogen concentrations of *Physcia adscendens* and *Hypogymnia physodes* in the 48 sampling sites of Grenoble area (the sites are ranked in three categories according to road exposure)

Categories (road exposure)	Sampling stations	Nitrogen concentration mean \pm standard deviation (%)	
		<i>Physcia adscendens</i>	<i>Hypogymnia physodes</i>
1	12	2.31 \pm 0.15	1.63 \pm 0.06
1	19	2.42 \pm 0.14	3.04 \pm 0.13
1	22		2.97 \pm 0.21
1	39	2.25 \pm 0.10	1.67 \pm 0.05
1	43	2.45 \pm 0.07	
2	1	2.58 \pm 0.09	3.19 \pm 0.06
2	2	2.97 \pm 0.08	3.28 \pm 0.14
2	3	2.90 \pm 0.05	1.66 \pm 0.02
2	4	1.75 \pm 0.07	
2	6	3.87 \pm 0.15	
2	7	3.06 \pm 0.12	1.42 \pm 0.01
2	8	3.93 \pm 0.13	
2	9	3.91 \pm 0.08	
2	10	2.35 \pm 0.08	
2	11	3.79 \pm 0.10	2.64 \pm 0.18
2	13	2.52 \pm 0.11	1.70 \pm 0.05
2	14	1.75 \pm 0.07	2.26 \pm 0.11
2	15	3.32 \pm 0.04	1.97 \pm 0.10
2	18	4.15 \pm 0.03	3.36 \pm 0.17
2	20	2.93 \pm 0.06	1.21 \pm 0.01
2	21	1.60 \pm 0.07	1.87 \pm 0.07
2	23		2.14 \pm 0.05
2	24	2.17 \pm 0.09	2.18 \pm 0.10
2	25	2.07 \pm 0.01	1.83 \pm 0.05
2	26		3.46 \pm 0.08
2	27	3.10 \pm 0.06	2.36 \pm 0.11
2	28	3.07 \pm 0.12	1.96 \pm 0.08
2	29	2.34 \pm 0.01	2.69 \pm 0.16
2	30	3.81 \pm 0.08	2.97 \pm 0.12
2	33	3.30 \pm 0.01	
2	34	3.10 \pm 0.03	
2	37	2.98 \pm 0.10	1.45 \pm 0.03
2	38		2.90 \pm 0.16
2	40	2.56 \pm 0.05	1.77 \pm 0.07
2	41	2.95 \pm 0.18	3.75 \pm 0.11
2	42	2.94 \pm 0.09	1.87 \pm 0.00
2	44	2.99 \pm 0.13	
2	46	2.51 \pm 0.13	2.87 \pm 0.18
2	47	3.55 \pm 0.04	3.54 \pm 0.06
2	48	3.13 \pm 0.15	
3	5	3.33 \pm 0.11	
3	16	3.34 \pm 0.08	2.35 \pm 0.12
3	17	3.73 \pm 0.13	
3	31	3.15 \pm 0.11	2.53 \pm 0.11
3	32	2.62 \pm 0.03	
3	35	3.27 \pm 0.05	2.22 \pm 0.04
3	36	3.45 \pm 0.15	3.06 \pm 0.05
3	45	4.21 \pm 0.12	

are generally higher than those quoted in the literature: from 0.42 to 2.6% for *H. physodes* (Hitch, 1971; Oksanen et al., 1990; Poikolainen et al., 1998) and from 1.0 to 1.3% for *P. adscendens* (Hitch, 1971). The urban environment of the Grenoble area appears to promote higher nitrogen contents in lichens, and *P. adscendens* shows significantly higher concentrations than *H. physodes* (Student *T*-test, $T^{***} = 3.66$, $P < 0.001$).

The influence of roads on lichen nitrogen concentrations was estimated. For this, a subjective assessment of the road size and the traffic flow was made. Three broad categories were used (Table 1): category 1 defined by small roads with light traffic represents 10% of the stations, category 2 defined by roads with a medium traffic flow (73%) and category 3 defined by roads with a heavy traffic flow (17%). The results indicate that roads have no influence on the nitrogen concentrations of the acidiphytic lichen *H. physodes*, but have an impact on the nitrogen concentrations of the nitrophytic *P. adscendens*; the samples collected from category 3 stations showed significantly higher concentrations than the samples collected from category 1 stations (ANOVA, $F^* = 3.92$, $P < 0.05$). Thus, only *P. adscendens* appears to be influenced by roads in terms of its nitrogen

concentrations, and for this reason, only the nitrogen data for this lichen were mapped.

The distribution of the nitrogen concentration of *P. adscendens* in the Grenoble area (Fig. 2) reveals that levels are higher in the center of the area characterized by dense traffic and along motorways located around the city. Along motorways, the stations in the west and south of the area show higher nitrogen levels than stations located in the north and east. Moreover, the two stations showing the highest nitrogen concentrations are located close to two factories emitting NO₂: a cement factory in the north-east of the area (site 45) and a chemical plant in the south-east of the area (site 18) (Calvo, 1992).

The mapping study showed the influence of roads on the nitrophytic lichen only. Consequently, the two transect studies were carried out to assess more accurately the spatial distribution of nitrogen concentrations of *P. adscendens* in relation to major roads.

3.2. Transects

For transect 1, the traffic index values range between 0 and 2.95 (Table 2), and for transect 2, the values range from 0.52 to 7.51 (Table 3). The significantly higher

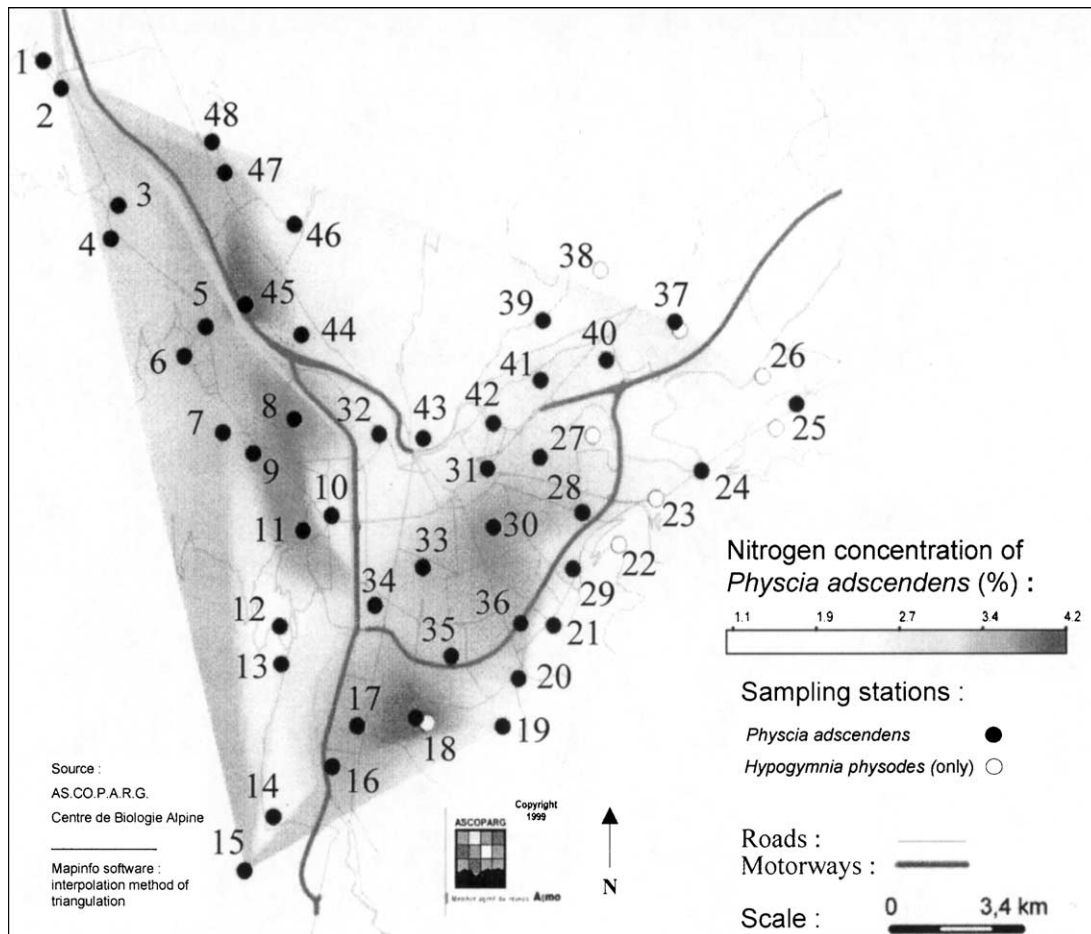


Fig. 2. Map of nitrogen concentrations of *Physcia adscendens* in the Grenoble area.

Table 2

Transect 1: Traffic index values and nitrogen concentrations of *Physcia adscendens* in July (unwashed samples) and in November (unwashed and washed samples)

Stations	Altitude (m)	Distance from MW (m)	Traffic index	Nitrogen concentration (%)					
				July		November			
				Unwashed		Unwashed		Washed	
				Mean	S.D.	Mean	S.D.	Mean	S.D.
1	220	300	0.43	3.14	0.05	3.22	0.13	3.01	0.14
2	220	305	0.42	2.89	0.02	2.82	0.09	2.85	0.16
3	225	387	0.63	2.84	0.03	2.64	0.11	2.77	0.09
4	225	450	1.11	2.86	0.03	3.13	0.04	2.72	0.12
5	225	562	1.46	2.78	0.07	3.18	0.11	3.03	0.15
6	225	612	1.20	2.56	0.05	2.69	0.08	2.40	0.10
7	230	725	0.36	1.80	0.05	1.91	0.10	1.87	0.07
8	235	912	0.07	2.52	0.02	2.56	0.06	2.16	0.08
9	235	937	0.11	2.60	0.06	2.34	0.06	2.43	0.08
10	250	1250	2.95	3.35	0.05	3.35	0.09	3.05	0.37
11	285	1512	1.39	1.70	0.09	1.85	0.04	2.14	0.04
12	285	1550	1.51	3.04	0.08	2.91	0.09	2.73	0.17
13	330	1975	0.00	1.64	0.13	1.53	0.03	1.49	0.17
Mean	245	883	0.90	2.59		2.62		2.51	
S.D.			0.83	0.55		0.57		0.48	

values for transect 2 (Mann–Whitney, $U^* = 45$, $P < 0.05$) can be explained by the lower average distance of stations from the motorway (352 m) than for transect 1 (883 m). Lichen nitrogen concentrations are also higher for transect 2 (Table 3) than for transect 1 (Table 2), in both July (Mann–Whitney, $U = 57.5$, $P < 0.05$) and November (Mann–Whitney, $U = 54$, $P < 0.05$), but the differences are not significant.

On both transects, the average nitrogen concentrations of *P. adscendens* are not significantly different between July and November respectively $2.59 \pm 0.55\%$ and $2.62 \pm 0.57\%$ (Mann–Whitney, $U = 80$, $P < 0.05$) on transect 1 (Fig. 3 and Table 2) and $3.01 \pm 0.84\%$ and $3.09 \pm 0.65\%$ (Mann–Whitney, $U = 83$, $P < 0.05$) on transect 2 (Fig. 4 and Table 3). Moreover, nitrogen concentrations in July and November are significantly correlated for transect 1 (Spearman's rank correlation, $r'^{***} = 0.90$, $P < 0.001$) and for transect 2 ($r'^{***} = 0.90$, $P < 0.001$). Washed samples of *P. adscendens* collected on transect 1 (November) show significantly lower nitrogen concentrations than unwashed samples (Wilcoxon, $W^* = 71.5$, $P < 0.05$) (Table 2).

Both transects show a positive correlation between the lichen nitrogen concentrations and the calculated traffic index: for transect 1 for November (Spearman's rank correlation, $r'^* = 0.62$, $P < 0.05$) and for transect 2 for both July (Spearman's rank correlation, $r'^{**} = 0.71$, $P < 0.01$) and November (Spearman's rank correlation, $r'^{**} = 0.72$, $P < 0.01$). Correlations for July for transect 1, however, are nearly significant (Spearman's rank correlation, $r' = 0.52$, $P < 0.1$).

Table 3

Transect 2: Traffic index values and nitrogen concentrations of *Physcia adscendens* in July and in November (unwashed samples)

Stations	Altitude (m)	Distance from SM (m)	Traffic index	Nitrogen concentration (%)			
				July—unwashed		November—unwashed	
				Mean	S.D.	Mean	S.D.
1	220	687	0.53	1.69	0.13	2.02	0.05
2	220	625	1.01	1.53	0.09	2.56	0.06
3	220	537	1.49	2.71	0.03	2.94	0.09
4	220	487	1.42	2.91	0.04	2.69	0.08
5	220	362	0.57	2.34	0.01	2.81	0.10
6	220	350	0.52	3.13	0.05	2.83	0.10
7	220	250	0.89	3.59	0.02	3.39	0.21
8	220	212	1.69	3.45	0.01	3.08	0.05
9	220	175	2.95	3.88	0.09	4.12	0.11
10	220	100	7.51	4.36	0.05	4.22	0.22
11	220	225	2.82	3.72	0.04	3.34	0.21
12	220	250	2.28	3.35	0.03	3.72	0.06
13	220	312	1.31	2.45	0.10	2.46	0.21
Mean	220	352	1.92	3.01		3.09	
S.D.			1.86	0.84		0.65	

4. Discussion

Our results show that nitrogen concentrations of *P. adscendens* depend on road traffic and road vicinity, but this is not true for *H. physodes*. These two species are defined according to the generally accepted ecological classification as “nitrophytes” and “acidiphytes” (Barkman, 1958; Wirth, 1980; Brand et al., 1988). They are used to characterize well-defined phytosociological lichen communities depending mainly on bark pH and light characteristics (*Physcietum ascendens*, Ochsner, 1928; *Parmelietum furfuraceae*, Hilitzer, 1925). At present, these epiphytic communities are ill-defined in urban areas (Khalil and Asta, 1998; Gombert, 1999).

In recent decades, urban pollution has been modified: with decreasing sulfur dioxide, other urban pollutants, mainly emitted by road traffic (NO_x) or emerging with secondary processes (O_3), are more pronounced. The effect of nitrogen oxides on lichens remains poorly understood because of their conflicting influence on bark characteristics. Whereas strong positive correlations exist between NO_2 and SO_2 concentrations, leading to acidification of bark (Van Dobben and Ter Braak, 1999), a recolonization of sensitive species to SO_2 levels (Seaward and Letrouit-Galinou, 1991; Seaward, 1993) as well as an increase of nitrophytic species has been observed in urban areas. A diachronic study carried out in the city of Lyon between 1984 and 1996 has shown an increase in acidiphytic and neutrophytic species (*Parmelia caperata*, *P. glabrata*, *P. tiliacea* and *Usnea* sp.) at the same time as an increase in nitrophytic species

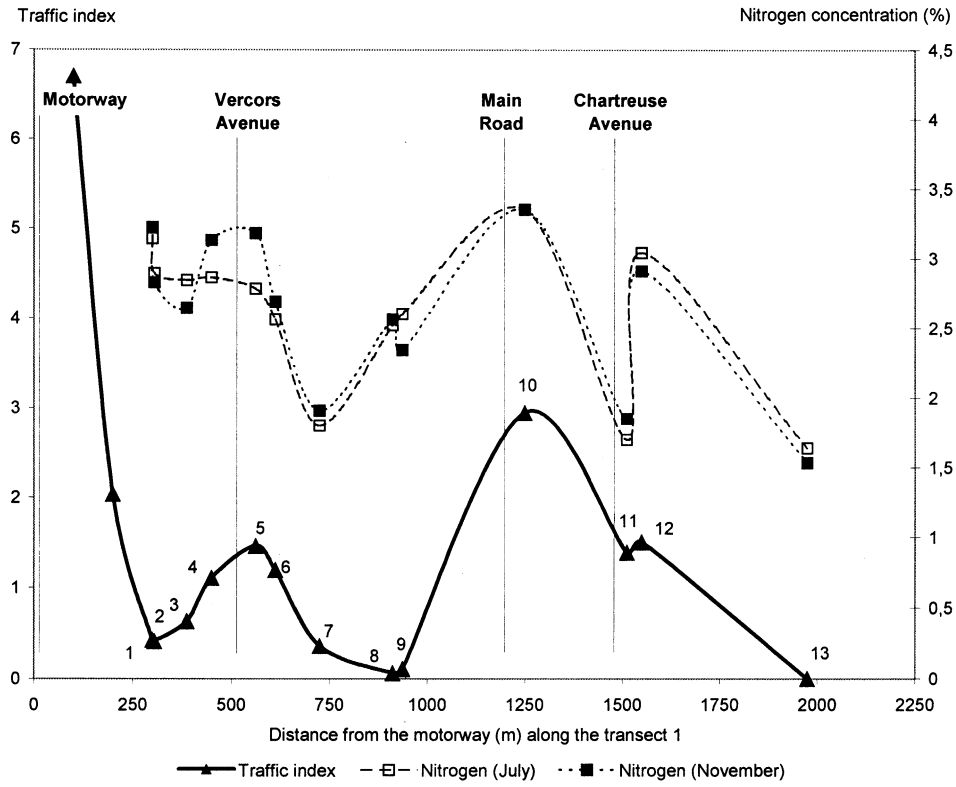


Fig. 3. Transect 1: Traffic index and nitrogen concentrations of *Physcia adscendens* (unwashed samples) in July and November.

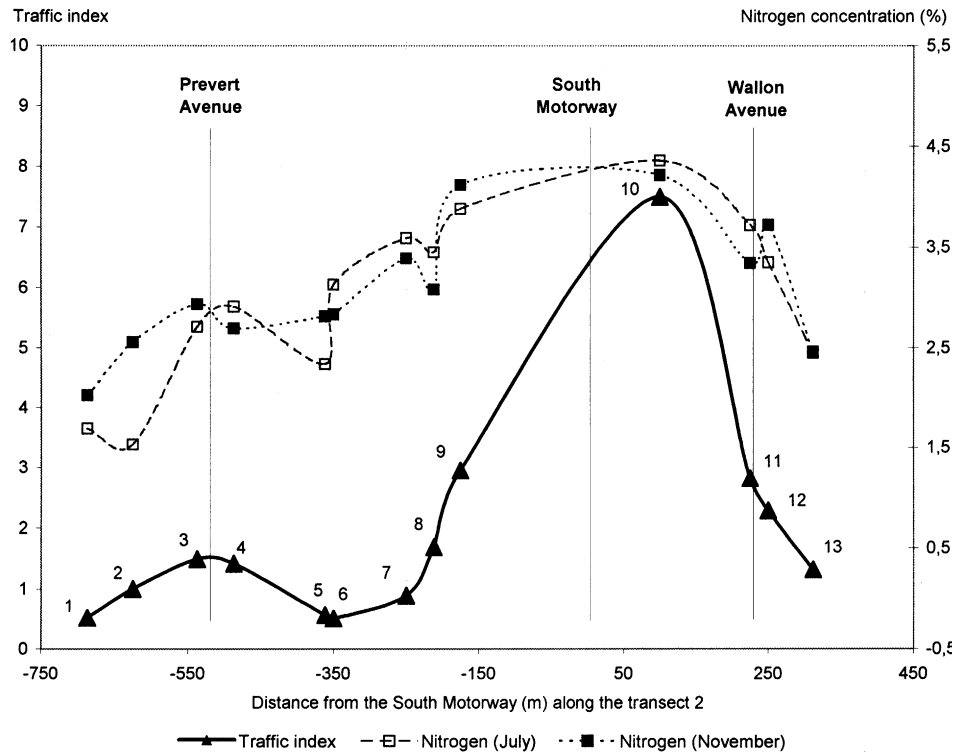


Fig. 4. Transect 2: Traffic index and nitrogen concentrations of *Physcia adscendens* (unwashed samples) in July and November.

such as *Candelariella xanthostigma*, *Physcia adscendens*, *Physconia grisea*, *Xanthoria parietina* and *X. polycarpa* (Khalil and Asta, 1998).

The influence of NO_x on particular lichen species is difficult to determine even if a general influence on lichen nitrophytic communities seems to be established. One reason is the numerous environmental factors other than air pollution levels which can play a role in the location, frequency and nutrition of a species. Hence, these factors have to be standardized between sampling sites. Since lichens grow on different tree species in urban areas, it is important to collect lichens from tree bark with similar chemical properties. Therefore only trees with sub-neutral barks were used in this study (poplar, maple, ash and walnut) according to the VDI guidelines (VDI 3799, 1995). The sampling sites were also characterized by few variations of climatic and topographic factors. All sites were located in an open urban environment (drier than rural areas) subjected to the same general direction of winds, where the altitude varies from 220 to 330 m. Hence, only lichens influenced by the road vicinity and by traffic exposure were collected.

Where road exposure at various sites is similar, but lichen nitrogen concentrations vary according to the species, different morphological and/or physiological properties of the lichen species might be responsible; for example, the rough thallus of *P. adscendens* may permit better entrapment of nitrogen particulates than the smooth thallus of *H. physodes*. Some studies have shown that for higher plants, the nitrate-reductase activity could be induced by NO_x (Norby et al., 1989; Thoene et al., 1991; Pearson and Stewart, 1993). Experiments carried out on nitrate-reductase activity of lichens (for in vivo conditions) revealed that nitrophytic lichens (*P. adscendens*, *Physconia grisea* and *Xanthoria parietina*) showed a detectable constitutive nitrate-reductase activity, whereas acidiphytic lichens (*Usnea* sp.) did not (Gombert, 1999; Gombert and Asta, 2000).

The statistical test indicates that washing removes nitrogen, which is certainly a part of nitrogen deposited on the surface of thalli (fine particles adsorbed by the lichen surface and gaseous nitrogen compounds, such as HNO_3 , which may be adsorbed but not assimilated). However, the washing method cannot be used to quantify the amount of nitrogen adsorbed and the part actually assimilated. Rainfall and subsequent leaching in natural conditions should be considered; even if there is a positive correlation between washed samples of *P. adscendens* and traffic index (Spearman's rank correlation, $r'^* = 0.58$, $P < 0.05$), it is better to collect samples during dry periods.

Samples of *P. adscendens* collected at two different periods (July and November) showed no variation in their nitrogen concentrations. For poikilohydric lichens and mosses, nutrient uptake is greatest during wet periods

(high moisture levels and rainfall). For mosses, growth rate tends to be seasonal and nitrogen measurements have to be made at the same stage in growth (Pitcairn et al., 1995). Nitrogen concentrations in some higher plants related to nitrogen atmospheric inputs also depend on age and season (Pitcairn et al., 1995). In contrast, for lichens, growth rates are too low to have any impact on nitrogen concentrations over the year. Moreover, nitrogen concentrations of long-lived lichens represent nitrogen which has been accumulated for many years. Some authors have shown that heavy metals are concentrated in the inner zones of lichen thalli, which are also the older parts (Bargagli et al., 1987). Differentiating growth parts is difficult, therefore whole lichen thalli are generally collected in bioaccumulation studies, and sampling can occur at anytime during the year.

The influence of road exposure on lichens was assessed by the application of a traffic index, which can be considered as a model. The positive correlations observed between the traffic index and nitrogen concentration of *P. adscendens* along the two transects confirm our first hypothesis that the concentrations are related to traffic exposure. Therefore a relationship between nitrogen oxides emitted by traffic and lichen nitrogen concentrations can be assumed. It should be noted that this traffic index has not been proved in relation to actual nitrogen deposition. Monitoring of nitrogen oxide levels by passive diffusion tube samplers at the sampling sites could have validated this, albeit for only short periods of time. To obtain a reliable suitable model for the spatial distribution of the effluents, it would be necessary to repeat such measurements several times. In contrast, bioindicators such as lichens integrate levels of elements in space and time. However, lichen nitrogen concentrations give relative spatial results for comparative purposes only. To obtain absolute data relative to pollutant levels, it is necessary to make experiments in fumigation chambers. Another way to prove the proposed model is to consider the bioaccumulation of some heavy metals (for instance lead and zinc) known to be useful markers for traffic flow (Pearson et al., 2000). On the growing evidence that NO_x and NH_x have different natural stable isotope signatures ^{15}N ($^{15}\text{N}/^{14}\text{N}$) (Heaton et al., 1997), Pearson et al. (2000) have shown a very good correlation between traffic exposure and ^{15}N of different mosses. It can be assumed that it would be the same for lichens.

In recent decades, several methods have been proposed for assessing environmental quality of urban areas on the basis of lichen data (Seaward, 1989). Some methods are based on biodiversity in relation to one single pollutant like SO_2 (Hawksworth and Rose, 1970), or several pollutants (Ammann et al., 1987), or no pollutant in particular (Leblanc and De Sloover, 1970; VDI, 1995; Nimis, 1999). The most recent methodology

is strongly standardized to provide easier comparisons throughout Europe (Asta et al., 2002). Numerous other methods are based on bioaccumulation of pollutants (e.g. heavy metals, radioelements, fluorides, pesticides, PAH) in lichens. Studies based on nitrogen bioaccumulation in lichens in rural areas are generally related to ammonia emissions (Van Herk, 2001). The present study is the first dealing with nitrogen concentrations in lichens which are related to traffic flow. Such monitoring could provide a useful marker for traffic flow and support the role of lichens as monitors of environmental and human health.

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