Ann. Bot. Fennici 34: 27–34
Helsinki 7 March 1997
ISSN 0003-3847

Chemical response of the lichens *Parmotrema austrosinense* and *P. conferendum* transplanted to urban and non-polluted environments

Martha S. Cañas*, Liliana Orellana & María L. Pignata

Cañas, M. S. & Pignata, M. L., Cátedra de Química General, Facultad de Ciencias Exactas, Físicas y Naturales, Universidad Nacional de Córdoba, Av. Vélez Sársfield 299, Córdoba 5000, República Argentina
Orellana, L., Instituto de Cálculo, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Pabellón No. 2, Ciudad Universitaria, Núñez 1428, Buenos Aires, República Argentina

Received 6 August 1996, accepted 20 October 1996

Thalli of the lichens *Parmotrema austrosinense* (Zahlbr.) Hale and *P. conferendum* Hale were taken from a non-polluted area and transplanted within this area, and to a downtown site in Córdoba, Argentina. Pigments, malondialdehyde, hydroperoxy-conjugated dienes and sulphur were measured after various exposure periods. Changes in the quantified compound concentrations were observed throughout the exposure period in both species and in both sites. In the urban site, however, chlorophyll concentration was lower and, in *P. conferendum*, malondialdehyde concentration was significatively higher. These results can be attributed to the effect of atmospheric pollutants on the transplanted lichens.

Key words: hydroperoxy-conjugated dienes, lichen, malondialdehyde, pigments, sulphur

INTRODUCTION

Lichen species show differing degrees of sensitivity to air pollution, but are generally affected adversely by sulphur dioxide, heavy metals, fluorides, ozone, nitrogen oxides and peroxyacetyl nitrate (PAN). Since the mixture of air pollutants arising from industrial areas and many cities is complex, it is difficult to identify the component(s) primarily responsible for the damage of lichens (Matthes & Feige 1983).

It has been shown that bleaching of lichen thalli due to chlorophyll degradation is one of the obvious signs of lichen damage as a consequence of airborne pollutants (Puckett *et al*. 1973, Showman 1975, Eversman 1978). However, symptoms of damage to cell membranes of either the lichen mycobiont or the photobiont partner, or both, are

* Fellow of Consejo de Investigaciones Científicas y Tecnológicas de la Provincia de Córdoba (CONICOR)

detectable in *Ramalina duriaei* transplanted to polluted sites long before any indication of damage becomes apparent in the photobiont chlorophyll (Garty *et al*. 1993).

Significant damage to lichen cell membranes has been shown to result from exposure to SO_2 (Puckett *et al*. 1977, Pearson & Henrikson 1981, Pearson & Rodgers 1982, Fields & St. Clair 1984). Furthermore, air pollutants such as O_3 and NO_2 are potent catalysts for the peroxidation of membrane lipids (Menzel 1976). In biological systems, the presence of oxidation products such as malondialdehyde is directly related to the beginning of peroxidation of unsaturated fatty acids (Mehelman & Borek 1987). Thus, the process of lipid peroxidation is accompanied initially by rearrangement of the double bonds in natural unsaturated fatty acids leading to diene conjugation (Slater 1972, Menzel 1976). All types of membrane are sensitive to oxidation processes generated by free radicals and nitrogen oxides. Among the atmospheric pollutants that could initiate such reactions are O_3 and SO_2 (Mead 1987). González and Pignata (1994) have noted an increase in malondialdehyde concentration in relation to sulphur content in *Punctelia subrudecta* when transplanted to a contaminated area. Furthermore, González *et al*. (1996) have observed higher concentrations of MDA and hydroperoxy conjugated dienes (HPCD) in *Ramalina ecklonii* transplanted at sites with combined conditions of medium or heavy traffic and heavy industries.

The aim of the present work was to study the chemical response of *Parmotrema austrosinense* (Zahlbr.) Hale and *P. conferendum* Hale transplanted during different exposure periods to a polluted site and to compare this with the chemical response of these lichens transplanted during the same exposure periods to a non-polluted site.

MATERIAL AND METHODS

Lichens and transplantation

Thalli of *Parmotrema austrosinense* and *P. conferendum* were collected from a "clean air" site near Villa General Belgrano, southwest Córdoba, Argentina. Part of this freshly picked material was subjected to the same chemical analysis carried out on the transplanted material, so as to obtain a baseline level for the sampling.

Lichen-bags were prepared by weighing 12 g of fresh material, and packed loosely in a fine nylon net. On August 1st 1994, half of the lichen-bags were transplanted onto trees at a site near a place where the lichens were collected (non-polluted site), and the other half were placed 5 m above the ground on a balcony in downtown Córdoba where there is a high traffic level (urban site).

September, October, December 1994, and February 1995 corresponded to the end of each exposure period (one, two, four and six months). Each sample (one sample per site, each consisting of several thalli) was carefully rinsed in order to eliminate dust deposited on the thallus surface. The thalli were dried and shredded (using a blender with a blade) to achieve homogeneity, and then freeze-dried.

Transplantation sites

Córdoba city with a population of 1 189 000 inhabitants (according to a 1991 census) was selected as an atmospherically polluted site, and is located in the centre of the Argentine Republic, 31°24´S, 64°11´W. The city is 440 m a.s.l. and has an irregular topography. Its general structure is funnel-shaped with a positive slope from the centre towards the surrounding areas. This somewhat concave formation reduces air circulation and causes frequent thermal inversions in autumn and winter. The climate is sub-humid, with an average annual precipitation of 790 mm, concentrated principally in summer. Mean annual temperature is 17.4°C and prevailing winds come from the NE and SE.

Córdoba is one of the most polluted cities in the country. Emission of air pollutants increased by 50% between 1973 and 1983, reaching 287 metric tons of total pollutants/day (Servicio Meteorológico Nacional de la República Argentina 1986). There are no data on the quantity or quality of pollutants discharged over the last 12 years, but all indicators point to a worsening situation. Smog clouds occur more frequently and for longer periods than ever before. The level of pollutants in the atmosphere is already high enough to be a health threat.

The "non-polluted" site is situated 70 km SW of Córdoba in a tectonic valley; it is 1 000 m a.s.l., 31°58´S, 64°34´W. The weather is sub-humid, with an average annual precipitation of 850 mm, concentrated principally in summer. Mean annual temperature is 13°C and prevailing winds are from the NE and SE.

Climatological data on both urban and non-polluted sites during the research period are shown in Table 1.

Pigments

One hundred milligrams of lichen material was ground with glass powder into a mortar and then homogenized in 10 ml of ETOH at 96% v/v. Subsequently, the supernatant was separated. Absorbance (665, 649 and 470 nm) was measured with a spectrophotometer Beckman DU 7000, and

chlorophylls (Chl-*a*, Chl-*b* and total Chl as Chl-*a* + Chl-*b*) and carotenoid concentrations were calculated on a dry weight basis (Lichtenthaler & Wellburn 1983). An extraction by ethanol was used because it was established in previous tests that in the quantification there were no interferences with lichen compounds abundant in this genus (Ahmann & Mathey 1967) and which are very soluble when acetone is used as the extracting solvent (Elix *et al*. 1988). Simultaneous extraction of lichen compounds could give erroneous results for chlorophyll quantification due to an increase in phaeophytinization by these compounds (Brown & Hooker 1977).

Peroxidation product estimation

Malondialdehyde (MDA) was measured by a colorimetric method (Heath & Packer 1968). The amount of MDA present was calculated from the extinction coefficient of 155 mM^{-1} cm^{-1} (Kosugi *et al*. 1989). Results were expressed in µmol g^{-1} d. wt.

Hydroperoxy-conjugated dienes (HPCD) were extracted by homogenization of the lichen material in 96% v/v ethanol at a ratio of 1:50 f. wt/v with an Ultra Turrax homogenizer. The absorption was measured in the supernatant at 234 nm and its concentration was calculated by means of $\varepsilon = 2.65 \times 10^4$ M^{-1} cm^{-1} (Boveris *et al*. 1980). Results were expressed as nmol g^{-1} d. wt.

Sulphur content

Five millilitres of $Mg(NO_3)_2$ saturated solution was added to 0.5 g of lichen and dried in an electric heater. Subsequently, the sample was heated in an oven for 30 min at 500°C. The ashes were then suspended in 10 ml of 6 M HCl, filtered, and the resulting solution boiled for 3 min. The solution was brought to 50 ml with distilled water.

The amount of SO_4^{2-} in the solution was determined by the acidic suspension method with barium chloride (Toennies & Bakay 1953) which subsequently allowed for the calculation of sulphur content in each sample. The concentration was expressed in mg g^{-1} d. wt.

Statistical analyses

For each species a two way analysis of variance for each chemical variable was carried out. Two factors were considered, exposure period and transplantation site.

The proposed model for the observations of each variable was:

$$Y_{ij} = \mu + \alpha_i + \beta_j + \varepsilon_{ij}, \quad (1)$$

where: $i = 1, 2$ and $j = 1, \ldots, 4$

Y_{ij} is the mean of the three replications obtained from the lichen-bag subjected to the ith transplantation site during the jth exposure period, µ is the population mean, α_i is the fixed effect of the ith transplantation site, β_j is the fixed effect of the jth exposure period, ε_{ij} is the random error in the ith transplantation site and jth exposure period.

This model supposes no interaction between the main effects. Tukey's Test for non-additivity was employed to check this assumption.

The assumptions of the analysis of variance (constant variance and normal error distribution) were evaluated through graphic methods (residuals vs. fitted values, box-plots and stem-leaf plots).

RESULTS

Pigments

In both species Chl-*a* concentration decreased both in the non-polluted and in the urban site during

Table 1. Monthly temperature (°C) at the two transplantation sites and monthly rainfall (mm) in Córdoba city during the study period.

	Non-polluted site (Villa General Belgrano)				Urban site (Córdoba city)				
	Temperature				Temperature				Rainfall
	Mean min.	Mean max.	Mean	No. of days < 0°C	Mean min.	Mean max.	Mean	No. of days < 0°C	
August	4.2	15.5	9.9	3	7.8	19.9	13.8	0	45.7
September	6.3	18.4	12.3	5	11.6	24.7	18.1	0	7.2
October	8.8	18.2	13.5	0	13.4	24.2	18.8	0	64.2
November	11.7	23.8	17.8	0	15.6	27.9	21.7	0	101.6
December	15.1	26.5	20.8	0	20.4	33.0	26.7	0	59.0
January	13.6	23.4	18.5	0	18.0	29.0	23.5	0	142.3
Mean ± *S.D.*	9.9 ± 4.2	21.0 ± 4.2	15.5 ± 4.2		14.5 ± 4.5	26.4 ± 4.5	20.4 ± 4.5		
Total				8				0	420.0

the first exposure months (August and September) as compared with basal condition (Fig. 1). This decrease was more evident in the urban site than in the non-polluted site. Chl-*b* and total Chl concentrations also decreased in *Parmotrema austrosinense* for both non-polluted samples as well as for urban samples. In *P. conferendum* these compounds decreased during the first months only for urban samples since an increase in Chl-*b* and total Chl concentrations, as regards the baseline value, was observed during the first exposure month in thalli transplanted to the non-polluted site in this species. Carotenoid content decreased in this site for both species during the first two months. The same was observed in urban samples of *P. austrosinense*, while in *P. conferendum* an increase in carotenoid content was observed in this site during the first exposure month.

From the fourth exposure month on (November, with mean maximun temperature over 25°C and heavier rainfall), both species showed an increase in the concentrations of Chl-*a*, total Chl and carotenoids, with this increase being higher in the non-polluted site. Chl-*b* showed a different variation, as in both species its concentration decreased from the fourth exposure month on, but this decrease was more evident in urban samples. In *Parmotrema conferendum*, an important decrease in Chl-*b* was observed during the sixth exposure month in the urban site.

Table 2 shows the results of the ANOVA for all the variables as *p*-values for *Parmotrema austrosinense* and *P. conferendum*.

In both species, a large increase in the carotenoid levels was observed for the longer exposure periods (they correspond to months with higher mean temperatures and humidity levels). However, no significant differences were found in the carotenoid concentration between the non-polluted and the urban site for either of the species.

Chl-*a* showed a similar variation pattern in both species and its concentration was significatively higher in lichens transplanted to the non-polluted site than in those transplanted to the urban site. In *Parmotrema austrosinense* a significant increase in Chl-*a* concentration was observed when the exposure period was prolonged.

Chl-*b* also showed a similar variation pattern in both species. Chl-*b* levels were higher in the non-polluted site than in the urban site, although this difference was not significant. Furthermore, in *Parmotrema austrosinense* a significant decrease in Chl b concentration was observed when the exposure period was prolonged.

The same variation pattern was observed for total Chl as was found for Chl-*a* in *Parmotrema austrosinense* with regard to transplantation site and exposure period. In contrast, in *P. conferendum* the statistical analysis revealed a significant interaction between the two factors (Table 2).

Table 2. Results of ANOVA for the chemical variables in *Parmotrema austrosinense* and *P. conferendum* (*p*-values in boldface show significant results of each test). Tukey's test for multiple comparisons is included where exposure period effect was significant ($p < 0.05$).

	Carotenoids	Chl-*a*	Chl-*b*	Total Chl	HPCD	MDA	Sulphur
P. austrosinense							
Transplantation site effect	0.481	**0.018**	0.187	**0.013**	0.570	0.182	0.498
Exposure period effect	**0.012**	**0.001**	**0.031**	**0.012**	0.245	0.628	0.294
Non-additivity test	0.590	0.440	0.930	0.410	**0.017**	0.710	**0.002**
Multiple comparison							
Tukey's test	1, 2 ≠ 4, 6	1, 2 ≠ 4, 6	1, 2 ≠ 4	1, 2 ≠ 6; 2 ≠ 4	–	–	–
P. conferendum							
Transplantation site effect	0.980	**0.038**	0.076	**0.025**	0.814	**0.006**	0.990
Exposure period effect	**0.047**	**0.012**	0.069	0.069	0.086	**0.009**	0.245
Non-additivity test	0.860	0.450	0.830	**0.040**	0.870	0.350	0.730
Multiple comparison							
Tukey's test	2 ≠ 6	2 ≠ 4, 6	–	–	–	1, 2 ≠ 6	–

≠ means significatively different exposure months.

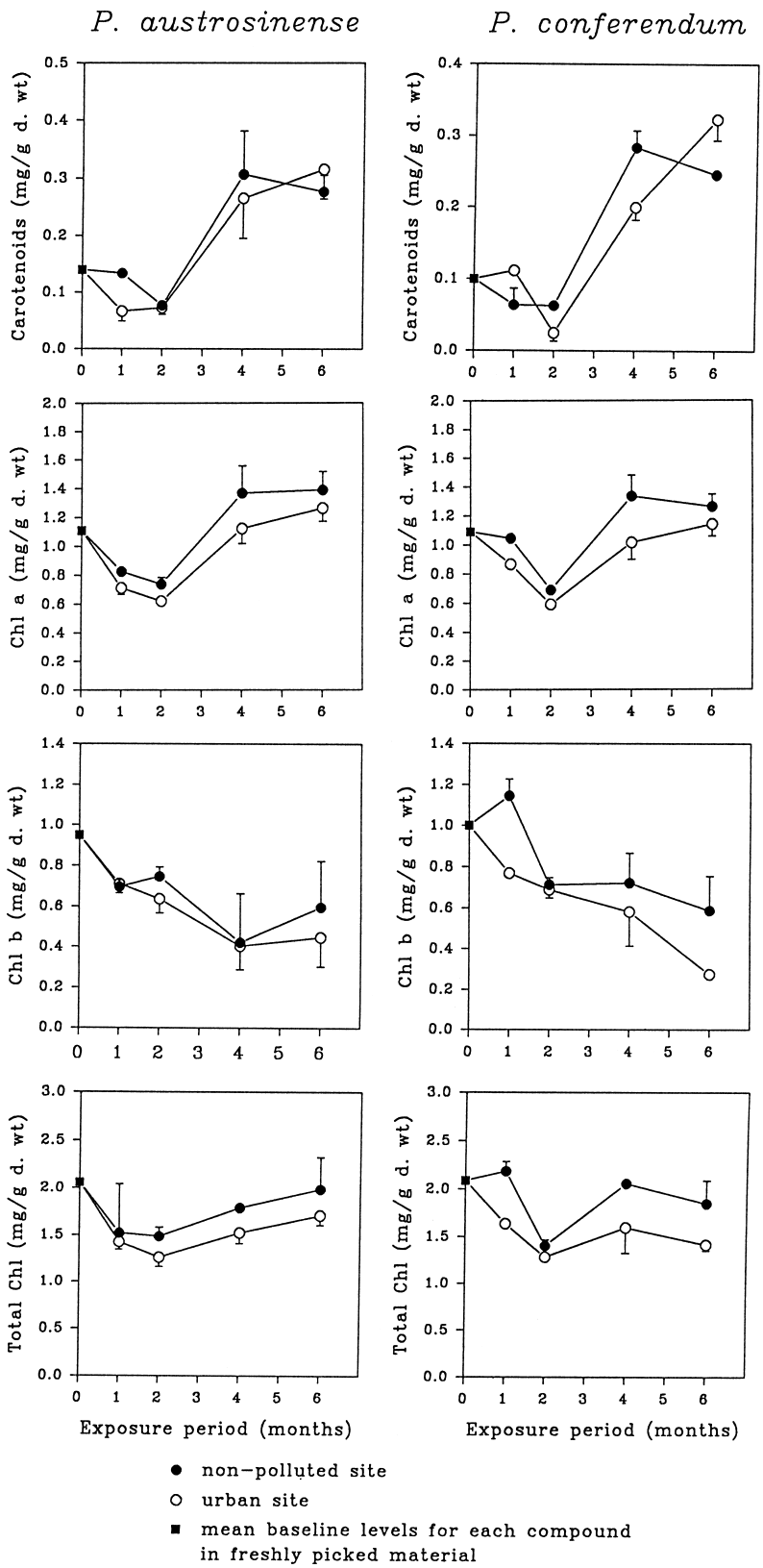

Fig. 1. Pigment concentrations (mean ± *S.E.* of 3 replicates) in *Parmotrema austrosinense* and *P. conferendum* after different exposure periods.

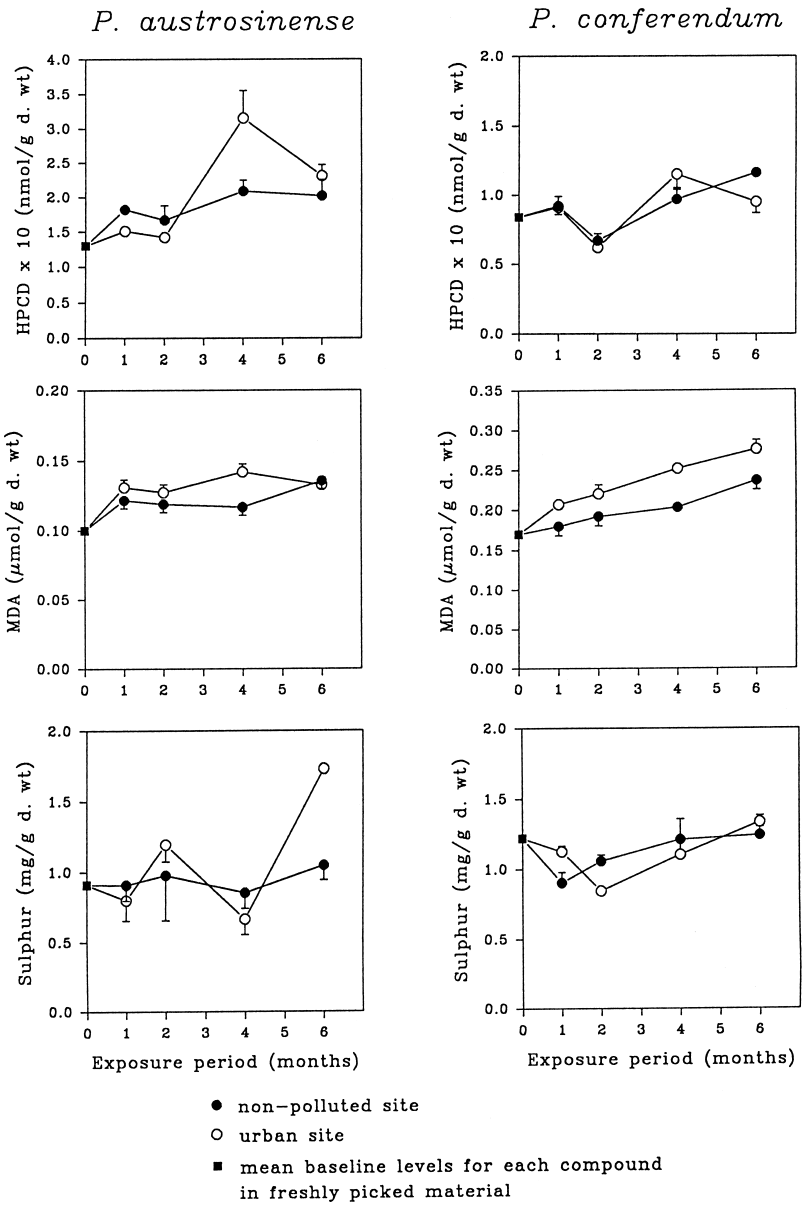

Fig. 2. Peroxidation products and sulphur content (Mean ± *S.E.* of 3 replicates) in *Parmotrema austrosinense* and *P. conferendum* after different exposure periods.

Sulphur and peroxidation products

In both species the sulphur concentration decreased slightly in the first exposure month in the non-polluted site as well as in the urban site (Fig. 2). However, while there was no important variation in lichens transplanted to the non-polluted site, in thalli of *Parmotrema austrosinense* transplanted to the urban site there was a large increase in sulphur concentration at the sixth exposure month. According to our observations and from the results of the statistical analysis (Table 2), the sulphur level of the studied species cannot be explained in relation to either transplantation site or the exposure period. The interaction between these factors was significant in *P. austrosinense* but not in the other species.

As regards MDA, *Parmotrema conferendum* showed an increase in its concentration throughout the study period (Fig. 2). This increase was higher in lichens transplanted to the urban site. In *P. austrosinense* an increase in MDA was only

observed during the first exposure month as compared with basal condition.

HPCD content did not show a regular variation pattern. The statistical analysis (Table 2) shows that the HPCD variation has no clear effect associated with either transplantation site or the exposure period. In *Parmotrema austrosinense* a significant interaction between both factors was observed (Fig. 2).

In *Parmotrema austrosinense*, MDA concentration did not show significant changes (Table 2). In contrast, in *P. conferendum*, MDA content was significatively higher in thalli transplanted to the urban site, and its level increased significatively when the exposure period increased (Fig. 2).

DISCUSSION

In both species, changes in pigment concentration were related to the transplantation site as well as to the exposure period. Significatively higher levels of Chl-*a*, total Chl and carotenoids were found in the fourth and the sixth exposure months as compared with the first and second months.

In general, comparison of samples transplanted to the non-polluted site vs. those to the urban site showed that pigments were sensitive to atmospheric pollutants. Throughout the study period, Chl-*a* and total Chl concentrations increased with the temperature increase. However, although in the urban site the temperature was higher during the study period, Chl-*a* and total Chl concentrations were lower. This lower value may be attributed to the presence of atmospheric pollutants in this urban site. Puckett *et al*. (1973), Showman (1975) and Eversman (1978) postulated that chlorophyll degradation is one sign of damage in lichens affected by atmospheric pollutants.

As regards sulphur accumulation, it was only observed to increase in *Parmotrema austrosinense* in the sixth exposure month and did not correlate either with a decrease in pigment content, as mentioned by other authors (Nash 1973, Ahmadjian 1993), or with an increase in membrane lipids peroxidation products, as mentioned by Pearson and Henrikson (1981) and González and Pignata (1994).

In relation to HPCD, it is interesting to note that during the first exposure months HPCD concentration was higher in lichens transplanted to non-polluted sites. This increase may have been caused by the lower temperature (< 0°C) samples were exposed to, since there is considerable evidence to suggest that membrane lipids are the primary sites to frost sensitivity (Evans & Ting 1973, Wilson 1978, Heath 1980). During warmer months (the fourth and sixth exposure month), HPCD concentration in urban samples was higher, probably due to the effect of air pollutants, as found by Levin and Pignata (1995) for *Ramalina ecklonii* transplanted in polluted sites of Córdoba.

In *Parmotrema conferendum*, MDA concentration was significatively higher in thalli transplanted to the urban sites. This could be evidence of damage to the lichen cell membranes due to oxidant atmospheric pollutants (Menzel 1976, Mehelman & Borek 1987).

In urban samples of *Parmotrema conferendum*, the MDA increase was significantly correlated with the Chl-*b* decrease throughout the study period ($R^2 = 0.914$; $p < 0.05$). This was not observed in the non-polluted samples. MDA has been mentioned as a sensitive parameter of atmospheric pollutants (González & Pignata 1994) and this result allows us to infer that the Chl-*b* decrease can be considered as a sign of damage caused by air pollutants in this species. De Koning and Jegier (1968) also observed in early studies of chlorophyll degradation in algae that Chl-*b* was considerably more sensitive to oxidant compounds than Chl-*a*.

The present results provide an insight to the chemical nature of *Parmotrema austrosinense* and *P. conferendum* and its relation to air pollutants. The quantified parameters are not enough to explain differences in sensitivity to air pollutants between the two studied species.

The present study helps to evaluate *Parmotrema austrosinense* and *P. conferendum* for biomonitoring programs in relation to atmospheric quality.

Acknowledgements. This research was supported by grants from CONICOR and SECyT. Thanks are extended to Dr. Mónica Adler and Cecilia Estrabou for identification of the lichens.

REFERENCES

Ahmadjian, V. 1993: The lichen symbiosis. — John Wiley & Sons Inc., USA. 250 pp.

Ahmann, G. B. & Mathey, A. 1967: Lecanoric acid and some constituents of Parmelia tinctorum and Pseudevernia intensa. — Bryologist 70: 93–97.

Boveris, A., Cadenas, E. & Chance, B. 1980: Low level chemiluminescence of the lipoxigenase reaction. — Photobiochem. & Photobiophys. 1: 175–182.

Brown, D. H. & Hooker, T. N. 1977: The significance of acidic lichen substances in the estimation of chlorophyll and phaeophytin in lichens. — New Phytol. 78: 617–624.

De Koning, H. W. & Jegier, Z. 1968: Quantitative relation between ozone concentration and reduction of photosynthesis of Euglena gracilis. — Atmospheric Environm. 2: 615–616.

Elix, J. A., Johnston, J. & Parker, J. L. 1988: A computer program for the rapid identification of lichen substances. — Mycotaxon 31: 89–99.

Evans, L. S. & Ting, I. P. 1973: Ozone induced membrane permeability changes. — Am. J. Bot. 61: 592–597.

Eversman, S. 1978: Effects of low-level SO_2 on Usnea hirta and Parmelia chlorochroa. — Bryologist 81: 368–377.

Fields, R. D. & St. Clair, L. L. 1984: A comparison of methods for evaluating SO_2 impact on selected lichen species: Parmelia chlorochroa, Collema polycarpon and Lecanora muralis. — Bryologist 87: 297–301.

Garty, J., Karary, Y. & Harel, J. 1993: The impact of air pollution on the integrity of cell membranes and chlorophyll in the lichen Ramalina duriaei (De Not.) Bagl. transplanted to industrial sites in Israel. — Arch. Environm. Contamin. Toxicol. 24: 455–460.

González, C. M. & Pignata, M. L. 1994: The influence of air pollution on soluble proteins, chlorophyll degradation, MDA, sulphur and amounts of heavy metals in a transplanted lichen. — Chem. & Ecol. 9: 105–113.

González, C. M., Casanovas, S. S. & Pignata, M. L. 1996: Biomonitoring of air pollutants from traffic and industries employing Ramalina ecklonii (Spreng.) Mey. and Flot. in Córdoba, Argentina. — Environm. Poll. 91: 269–277.

Heath, R. L. 1980: Initial events in injury to plants by air pollutants. — Annual Rev. Plant Phys. 31: 395–401.

Heath, R. L. & Packer, L. 1968: Photoperoxidation in isolated chloroplast. I. Kinetics and stoichiometry of fatty acids peroxidation. — Arch. Biochem. Biophys. 125: 189–198.

Henriksson, E. & Pearson, L. C. 1981: Nitrogen fixation rate and chlorophyll content of the lichen Peltigera canina exposed to sulfur dioxide. — Am. J. Bot. 68: 680–684.

Kosugi, H., Jojima, T. & Kikugawa, K. 1989: Thiobarbituric acid-reactive substances from peroxidized lipids. — Lipids 24: 873–881.

Levin, A. G. & Pignata, M. L. 1995: Ramalina ecklonii as a bioindicator of atmospheric pollution in Argentina. — Can. J. Bot. 73: 1196–202.

Lichtenthaler, H. K. & Wellburn, A. R. 1983: Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. — Biochem. Soc. Trans. 11: 591–592.

Matthes, U. & Feige, G. B. 1983: Ecophysiology of lichen symbiosis. — In: Lange, O. L., Nobel, P. S., Osmond, C. B. & Ziegler, H. (eds.), Physiological plant ecology, Vol. 3: 423–467. Springer, Berlin.

Mead, J. F. 1987: Free radical mechanism of lipid damage and consequences for cellular membrane. — In: Pryor, W. A. (ed.), Free radicals in biology: 76–122. Acad. Press, London.

Mehelman, M. A. & Borek, C. 1987: Toxicity and biochemical mechanisms of ozone. — Environm. Res. 42: 36–53.

Menzel, D. B. 1976: The role of free radicals in the toxicity of air pollutants (nitrogen oxides and ozone). — In: Pryor, W. A. (ed.), Free radicals in biology, Vol. 2.: 181–203. Acad. Press, New York.

Nash, T. H. 1973: Sensitivity of lichens to sulfur dioxide. — Bryologist 76: 333–339.

Pearson, L. C. & Henriksson, E. 1981: Air pollution damage to cell membranes in lichens. II Laboratory experiments. — Bryologist 4: 515–520.

Pearson, L. C. & Rodgers, G. A. 1982: Air pollution damage to cell membranes in lichens. III Field experiments. — Phyton (Austria) 22: 329–337.

Puckett, K. J., Nieboer, E., Flora, W. P. & Richardson, D. H. S. 1973: Sulphur dioxide: its effect on phytotoxicity. — New Phytol. 72: 141–154.

Puckett, K. J., Tomassini, F. D., Nieboer, E. & Richardson, D. H. S. 1977: Potassium efflux by lichen thalli following exposure to aqueous sulphur dioxide. — New Phytol. 79: 135–145.

Servicio Meteorológico Nacional de la República Argentina 1986: Boletines Informativos, 1–24.

Showman, R. E. 1975: Lichens as indicators of air quality around a coal-fired power generating plant. — Bryologist 78: 1–6.

Slater, T. F. 1972: Free radical mechanisms in tissue injury. — Pion, London. 47 pp.

Toennies, G. & Bakay, B. 1953: Photonephelometric microdetermination of sulphate and organic sulphur. — Anal. Chem. 25: 160–165.

Wilson, J. M. 1978: Lipid changes in plants at temperatures inducing chill-hardiness. — Pesticide Sci. 9: 173–183.