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Temporal Variation of Pigments and Peroxidation Products in the Lichen *Parmotrema uruguense* (Krempl.) Hale Transplanted to Urban and Non-Polluted Environments

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Abstract

Thalli of the lichen *Parmotrema uruguense* (Krempl.) Hale were taken from a non-polluted area and transplanted within this area and to two sites of Córdoba city (Argentina) with different traffic levels. Carotenoids, chlorophylls, hydroperoxy-conjugated dienes and malondialdehyde were measured after various exposure periods. For most of the quantified parameters, the temporal variation throughout the study period depended on the transplantation site of the lichens, this being more evident when the exposure period was prolonged. The lower content of carotenoids and Chl *a*, as well as the higher values of Chl *b*/Chl *a* ratio and hydroperoxy-conjugated dienes found in the downtown of Córdoba can be attributed to the effect of atmospheric pollutants on the transplanted lichens.

Keywords: Hydroperoxy-conjugated dienes, lichen, malondialdehyde, pigments

1. Introduction

Lichens are sensitive to air pollution and well suited as biological indicators for monitoring environmental quality (Ferry et al., 1973; Garty et al., 1985; Kauppi and Halonen, 1992; González and Pignata, 1994). However, different

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lichen species exhibit differential sensitivity to specific air pollutants (Nash, 1973; Ahmadjian, 1993).

Numerous experiments and field observations have shown a high sensitivity of many foliose and fruticose lichens to SO_2 (Balaguer and Manrique, 1991; Holopainen and Kärenlampi, 1984; Sanz et al., 1992). However, in regions with low SO_2 levels, other gaseous pollutants such as NO_x (Ross and Nash, 1983; Eversman and Sigal, 1987) and PAN become equally important (Sigal and Taylor, 1979; Nash and Gries, 1991). 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 to lichens (Matthes and Feige, 1983).

Several biological parameters have been used to assess the damage to lichens caused by pollution. Particularly important are the physiological and biochemical changes observed (James, 1973). It has been shown that bleaching of lichen thalli due to chlorophyll degradation is one the most obvious signs of lichen damage as a consequence of airborne pollutants (Puckett et al., 1973; Showman, 1975; Eversman, 1978). According to Senser et al. (1990) carotenoids seem highly susceptible to ozone-mediated photooxidation. Thus, besides their function as light-harvesting pigments that contribute to photosynthesis, carotenoids protect chlorophylls against oxidative destruction by O_2 under high light intensities (Siefermann-Harms, 1987).

Garty et al. (1993a) support the hypothesis that symptoms of damage to cell membranes of either the mycobiont or the photobiont partner, or both, are detectable in *Ramalina duriaei* (De Not.) Bagl. transplanted to polluted sites long before any indication of damage becomes apparent in the photobiont chlorophyll.

Significant damage to lichen cell membranes has been shown to result from exposure to SO_2 (Puckett et al., 1977; Pearson and Henrikson, 1981; Pearson and Rodgers, 1982; Fields and St. Clair, 1984). On the other hand, air pollutants (O_3, NO_2) are potent catalysts of the peroxidation of membrane lipids (Menzel, 1976). In biological systems, the presence of oxidation products such as malondialdehyde (MDA) is directly related to the beginning of peroxidation of unsaturated fatty acids (Mehelman and Borek, 1987).

Thus, the process of lipid peroxidation is accompanied, in the first step, by rearrangement of the double bonds in natural unsaturated fatty acids leading to the 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, ozone and sulphur oxides should be mentioned (Mead, 1987). In this sense, González and Pignata (1994) have noted an increase in MDA concentration in relation to sulphur content in *Punctelia subrudecta* (Nyl.) Krog. when transplanted to a

polluted area. Furthermore, González et al. (1996) have observed higher concentrations of MDA and hydroperoxy-conjugated dienes (HPCD) in *Ramalina ecklonii* (Spreng.) Mey. and Flot. transplanted to sites with combined conditions of medium or heavy traffic and heavy industries.

Recent studies have provided evidence for the interference of air pollutants in seasonal physiological and metabolic changes in lichens (Garty et al., 1993b). The aim of the present work was to study the temporal variation of chlorophylls, carotenoids, hydroperoxy-conjugated dienes and malondialdehide in *Parmotrema uruguense* (Krempl.) Hale transplanted for different exposure periods to non-polluted and urban environments.

2. Materials and Methods

Lichens and transplantation

Thalli of *Parmotrema uruguense* (Krempl.) Hale 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 July 1st 1994 nine lichen-bags were transplanted 3 m above the ground onto trees in a site near a place where the lichens were collected (non-polluted site); others were placed 3 m above the ground on a post in two sites of Córdoba city with different traffic levels (nine lichen-bags in each site, too).

In August, September and November 1994 that corresponded to the end of each exposure period (one, two and four months), three lichen-bags were taken down in each transplantation site. Thalli of each lichen-bag (each one with several thalli) were 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) is located in the centre of the Argentine Republic. The city is 440 m a.s.l., 31°24'S, 64°11'W 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 1973–1983, reaching 287 metric tons of total pollutants/day (Servicio Meteorológico Nacional de la República Argentina, 1986).

In Córdoba city two transplantation sites were selected according to their traffic level at peak time (10–12 a.m.). One of these urban sites was in a suburb of the city and was considered a "low polluted" site with less than 50 vehicles/h. The other site was downtown and was considered a "high polluted" site with more than 100 vehicles/h. In both urban sites, vehicular traffic consists of automobiles, public transit buses or diesel trucks carrying cargo.

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.

Table 1. Monthly temperature (°C) and total rainfall (mm) in the transplantation-site areas during the study period.

	Non-polluted area (Villa General Belgrano)			Urban area (Córdoba city)			
	Mean min. temp.	Mean max. temp.	Mean temp.	Mean min. temp.	Mean max. temp.	Mean temp.	Total rainfall
July	1.4	12.8	7.1	4.7	18.7	11.7	3.9
Aug.	4.2	15.5	9.9	7.8	19.9	13.8	45.7
Sept.	6.3	18.4	12.3	11.6	24.7	18.1	7.2
Oct.	8.8	18.2	13.5	13.4	24.2	18.8	64.2
Mean ± S.D. for the period	5.2±3.1	16.2±2.6	10.7±2.8	9.4±3.4	21.9±3.0	15.6±3.4	

Pigments

Lichen material (100 mg) was ground with glass powder in a mortar and then homogenized in 10 ml of ETOH at 96% v/v at room temperature. After 15 minutes the supernatant was separated. Absorbance (665, 649 and 470 nm) was measured with a spectrophotometer Beckman DU 7000. Chlorophylls (Chl *a* and Chl *b*) and carotenoid concentrations were calculated on a dry weight basis (Lichtenthaler and Wellburn, 1983). Ethanol extraction was used because it was established in previous tests that in the measurement of chlorophylls and carotenoids by this method, there were no interferences with lichen compounds abundant in this genus (Ahmann and Mathey, 1967), which are very soluble in acetone (Elix et al., 1988). Simultaneous extraction of lichen compounds can give erroneous results for chlorophyll quantification due to an increase in phaeophytinization by these compounds (Brown and Hooker, 1977).

Peroxidation product estimation

Malondialdehyde (MDA) was measured by a colorimetric method (Heath and 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 mmol 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. 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.

Water content

Due to the absence of precipitation data for the non-polluted site and taking into account that the water status of the thallus is necessary to interpret physiological processes, the water content was calculated.

Lichen material (1 g) was stored at 60±2°C until constant weight. Water content was calculated by difference between fresh weight (f. wt) and dry weight (d. wt) and was expressed as g g^{-1} f. wt.

Statistical analyses

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:

$$Y_{ijk} = \mu + a_i + \beta j + (a\beta)_{ij} + e_{ijk}$$

where
i = 1, 2, 3
j = 1, 2, 3
k = 1, 2, 3.

Y_{ijk} is the mean of the three replications obtained from the **kth** lichen-bag (experimental unit) subjected to the **ith** transplantation site after the **jth** exposure period; μ is the population mean; a_i is the fixed effect of the **ith** transplantation site; β_j is the fixed effect of the **jth** exposure period; $(a\beta)_{ij}$ is the interaction effect between the **ith** transplantation site and the **jth** exposure period; e_{ijk} is the random error in the **kth** lichen-bag subjected to the **ith** transplantation site after the **jth** exposure period.

In addition, one-way ANOVA was used, for each chemical variable, to evaluate transplantation site differences after each exposure period as well as exposure period differences at each transplantation site. Tukey's Test for multiple comparisons were made when appropriate.

3. Results

The two-way analysis of variance revealed that carotenoids, Chl *a*, Chl *b*/Chl *a* ratio, water content and MDA showed a significant interaction between the principal factors; Chl *b* concentration was affected by the exposure period only while HPCD showed effects associated to the exposure period as well as to the transplantation site (Table 2).

After one and two exposure months, carotenoid concentration of the lichens transplanted to the non-polluted site was similar to the basal condition (Fig. 1a). In the urban samples, lower carotenoid concentrations were observed after two exposure months than those after one exposure month, these differences being significant only for lichens transplanted to the low polluted site. After two exposure months, a significant lower carotenoid concentration in the urban samples was also observed. After four exposure months, the highest content of carotenoids was observed in all the transplantation sites with respect to the others exposure periods, and after this period, carotenoid concentration was significantly lower in thalli transplanted to the high polluted site.

In all the transplantation sites, Chl *a* concentration found after one exposure month was higher than the baseline level as well as than the concentration found after two exposure months. This difference was significant only for

Table 2. Results of two-way ANOVA for the chemical variables in *Parmotrema uruguense*.

	Transplantation site effect	Exposure period effect	Interaction effect	Multiple comparisons Tukey's Test
Carotenoids	**0.001**	**<0.001**	**<0.001**	–
Chl *a*	**0.001**	**<0.001**	**0.002**	–
Chl *b*	0.233	**<0.001**	0.717	1, 2 ≠ 4
Chl *b*/Chl *a*	**0.006**	**<0.001**	**0.006**	–
Water	**<0.001**	**<0.001**	**<0.001**	–
HPCD	**<0.001**	**<0.001**	0.087	1 ≠ 2, 4; *
MDA	**<0.001**	0.084	**0.008**	–

p-values in bold show significant results of each test. Tukey's Test for multiple comparisons is included where principal effects were significant ($p<0.05$). ≠ = significantly different exposure months. *The three transplantation sites were significantly different.

lichens transplanted to the low polluted site (Fig. 1b). No differences were observed among the transplantation sites after one and two exposure months. After four exposure month, the Chl *a* content of lichens transplanted to the non-polluted and to the low polluted sites was higher than that found after two exposure months, this difference being significant only for lichens transplanted to the non-polluted site. After this exposure period, significant differences in the Chl *a* content were observed among the transplantation sites.

Chl *b* concentration was similar to the basal condition after one and two exposure months (Fig. 1c). After four exposure months, Chl *b* content was lower than that found after the others exposure periods, although this difference was significant only for the urban sites.

In all the transplantation sites, the Chl *b*/Chl *a* ratio was similar to the basal condition after one exposure month (Fig. 1d). After two exposure months, the Chl *b*/Chl *a* ratio of the urban samples was higher than that after one exposure month, this difference being significant only for lichens transplanted to the low polluted site. After this exposure period, this parameter was significantly higher in the urban samples. After four exposure months, lower values of this parameter were found in all the transplantation sites with respect to the others exposure periods. After this period, Chl *b*/Chl *a* ratio was significantly higher in lichens transplanted to the high polluted site.

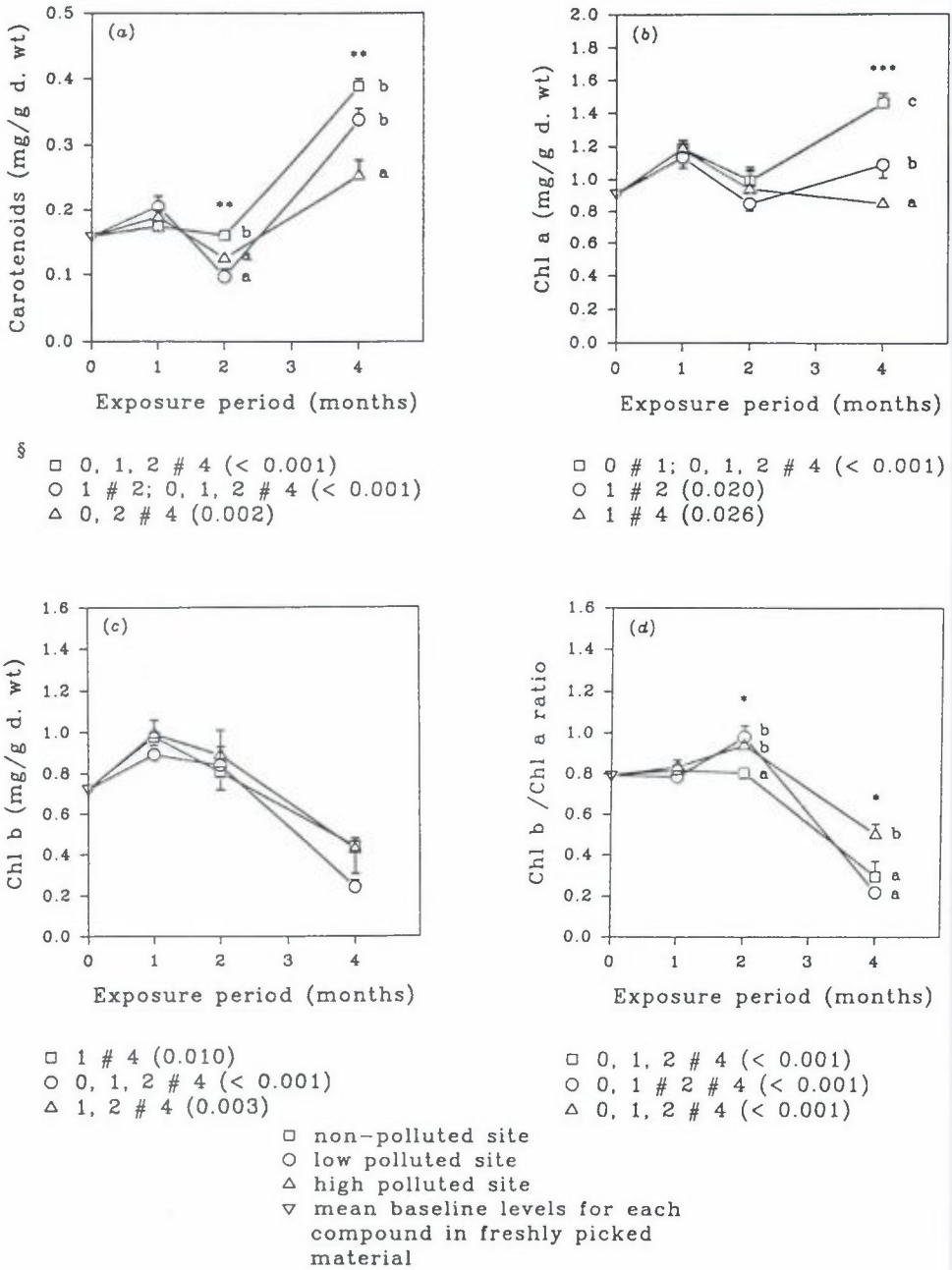

Figure 1. Pigments (Mean ± S.E., n = 3) in *Parmotrema uruguense* after different exposure periods. Monthly significant differences among transplantation sites are indicated with: *($p<0.05$), **($p<0.01$) and ***($p<0.001$). § = Comparison among exposure periods for lichens transplanted to each site. # = significantly different exposure months. p-values between brackets.

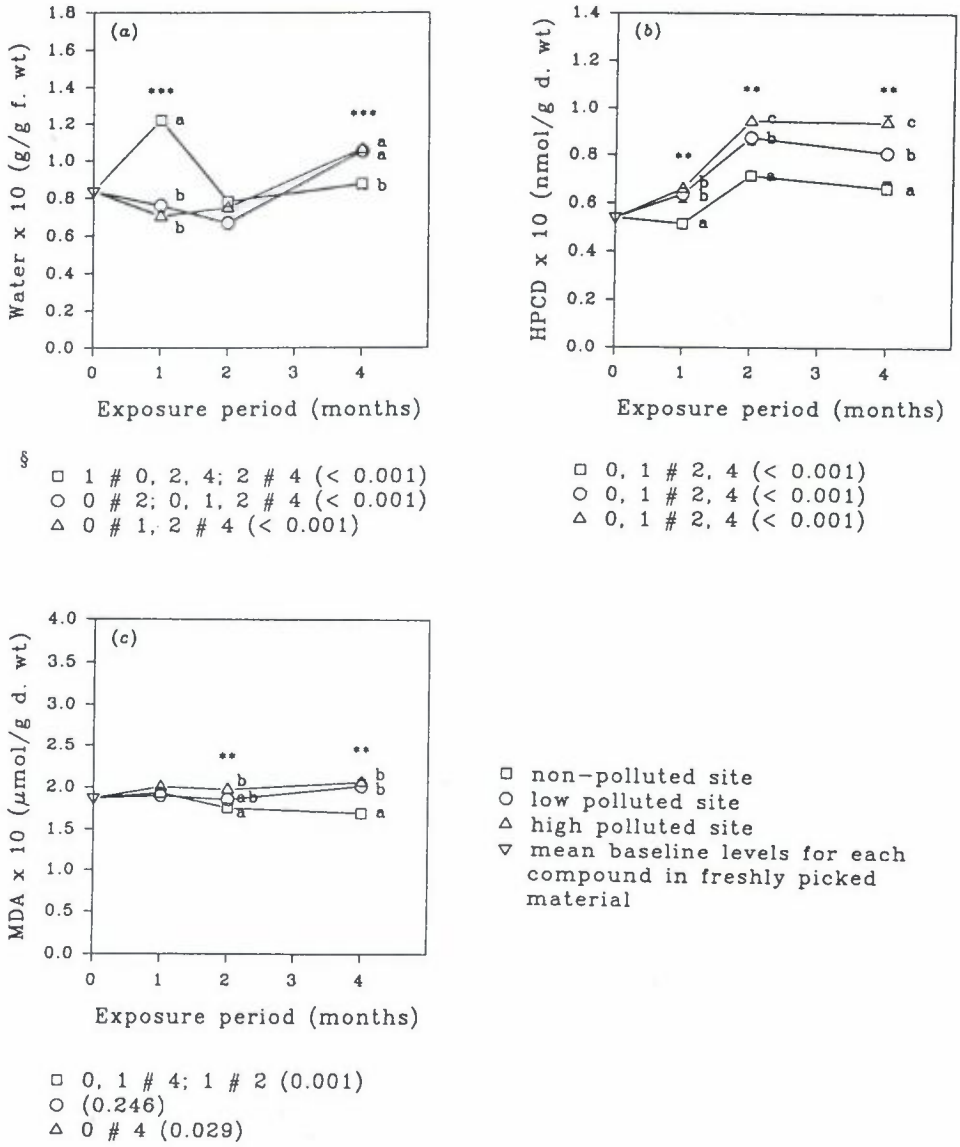

Figure 2. Water content and peroxidation products (Mean ± S.E., n = 3) in *Parmotrema uruguense* after different exposure periods. Monthly significant differences among transplantation sites are indicated with: *($p<0.05$), **($p<0.01$) and ***($p<0.001$). § = Comparison among exposure periods for lichens transplanted to each site. # = significantly different exposure months. p-values between brackets.

Throughout the study period, the variation of the water content was different in all the transplantation sites (Fig. 2a). Lichens transplanted to the non-polluted site showed significantly higher water content after one exposure month; and after four exposure months, significantly higher water content was found with respect to that found after two exposure months. The urban samples also showed high water content after four exposure months; but, after one exposure month, the values of this parameter in thalli transplanted to the low polluted site were similar to the baseline level, while in the high polluted site were lower. Significant differences between the non-polluted and urban sites were observed after one and four exposure months.

The variation of HPCD content throughout the study period was similar in all the transplantation sites (Fig. 2b). Thus, the HPCD content found after one exposure month was similar to the baseline level and significantly lower than the HPCD content found after the other exposure periods. Throughout the study period, the HPCD concentration was always significantly higher in lichens transplanted to the urban sites (Table 2). After two and four exposure months, significant differences were also observed between the urban sites, HPCD concentration being higher in thalli transplanted to the highly polluted site (Fig. 2b).

MDA content showed no important variation throughout the study period in any of the transplantation sites (Fig. 2c). After two and four exposure months, significant differences were observed between transplantation sites. Thus the MDA content of lichens transplanted to the highly polluted site was significantly higher than that transplanted to the non-polluted site after two exposure months. After four exposure months, significant differences between the non-polluted and the urban sites were observed, MDA being higher in the latter.

4. Discussion

For most of the quantified parameters, the temporal variation throughout the study period depended on the transplantation site of the lichens. For carotenoids and Chl *b*/Chl *a* ratio, differences between transplantation sites were observed after two and four exposure months. This may be due to different levels of atmospheric pollutants in each site. The decrease of carotenoid content in the presence of air pollutants has extensively been reported in higher plants (Sakaki et al., 1983; Senser et al., 1990; Mikkelsen et al., 1995; Carreras et al., 1996). Several pigment ratios have been used to evaluate the effect of air pollutants on plants: Cape et al. (1988) and Robinson and Wellburn (1991) proposed that a decrease in Chl *a*/Chl *b* ratio was a good indicator of foliar

damage in trees exposed to ozone. After four exposure months, carotenoid concentration and Chl *b*/Chl *a* ratio were similar in lichens transplanted to both the low polluted and non-polluted sites. Considering the differences in the environmental conditions between the urban and non-polluted sites, these results allow us to infer that these parameters are more affected by air pollutants than by the environmental conditions of the transplantation site.

For Chl *a* and water content, differences in the temporal variation between transplantation sites were observed early, after one exposure month. The lichens are poikilohydric organisms, whose water status varies passively with surrounding environmental conditions (Nash, 1996). They are highly dependent on precipitation, primarily in the form of rain (Ahmadjian, 1993). In the urban area, the higher precipitations occur in August and October (second and fourth exposure months, respectively) (Table 1). However, in *P. uruguense*, a high water content of thalli was observed after four exposure months, but not after two. This may be due to a different distribution of precipitations in each period. Lichens became desiccated relatively rapid (Ahmadjian, 1993). In August, almost all the rainfall was concentrated in one day, twenty days before the end of this exposure period. Thus, lichens underwent drying before samples were taken out. In October, it rained during several consecutive days, almost at the end of the exposure period. This gradual rehydration of the lichen without a subsequent desiccation resulted in a high water content of the thalli and allows lichens to recover their metabolic capacity (Ahmadjian, 1993; Nash, 1996). The correspondence between the water content in thalli and the metabolic capacity was evident in lichens transplanted to the non-polluted site, in which the highest Chl *a* concentrations were observed after one and four exposure months, when water contents of the thalli were also the highest. Green and Snelgar (1981) and Lange and Matthes (1981) mentioned that the net photosynthetic rate of lichens depended to a great extent to on the water content of their thalli. Matthes-Sears et al. (1987) found higher rates of net photosynthesis in lichens populations with more chlorophylls. In the urban samples there was no correspondence between chlorophylls and water content of the lichens. Thus, despite of the high water content of thalli transplanted to the urban sites after four exposure months, the chlorophyll concentration was low, specially in the high polluted site. This reflects an alteration of the normal metabolic processes of *P. uruguense* due to air pollutants. In lichens, transplanted samples generally show that chlorophyll degradation is positively correlated with pollution intensity (Garty et al., 1993a). In *P. uruguense*, this was evident after the most prolonged exposure period. This agreed with the studies by Showman (1972), who found damages to chlorophyll at high levels of sulphur dioxide, but only after some metabolic

processes such as photosynthesis have been affected. Sakaki et al. (1983) observed a similar response in ozone-fumigated plants.

Unlike Chl *a*, the Chl *b* content throughout the study period was not altered by the presence of air pollutants. Rao and LeBlanc (1965) and Gries (1996) noted that Chl *a* is more sensitive to oxidative attack than Chl *b*. However, in *P. conferendum*, a decrease in Chl *b* content due to the effect of air pollutants was observed (Cañas et al., 1997). There are no differences between the photobionts of *P. uruguense* and *P. conferendum* (Hale, 1983), that can explain these results.

A temporal variation of HPCD content independently of the transplantation site was observed, which probably reflects a seasonal variation of this parameters. However, a higher HPCD content was found at the high polluted site. An increase in the HPCD content in polluted sites was also observed by Levin and Pignata (1995) for *Ramalina ecklonii*. This could be evidence of damage to the lichen cell membranes due to oxidant atmospheric pollutants (Menzel, 1976; Mehelman and Borek, 1987).

MDA has been stated to be as a sensitive parameter for damage due to atmospheric pollutants (González and Pignata, 1994). However, no differences were found for *P. uruguense* between lichens transplanted to both urban sites. We cannot therefore confirm that the higher concentrations of MDA occur in urban samples due to air pollutants.

The present results provide an insight into the response of *P. uruguense* to the presence of air pollutants. Variation in the chemical parameters were observed in lichens transplanted from non-polluted to urban sites and suggest that *P. uruguense* may be useful in programs of atmospheric quality biomonitoring.

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