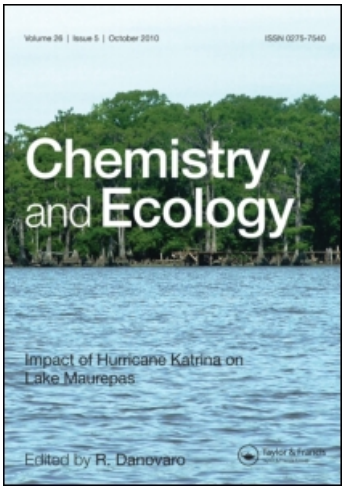

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THE INFLUENCE OF AIR POLLUTION ON SOLUBLE PROTEINS, CHLOROPHYLL DEGRADATION, MDA, SULPHUR AND HEAVY METALS IN A TRANSPLANTED LICHEN

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The lichen, *Punctelia subrudecta* (Nyl.) Krog., was transplanted to 10 biomonitoring sites during the period December 1990–March 1991. The total amounts of metals (Mn, Cu, Pb, Zn, Fe and Al) detected in the lichen thalli after the period of exposure, were compared with the chlorophyll degradation, amounts of sulphur, MDA concentrations and soluble protein concentrations in the same material. The MDA content was directly related to the amount of sulphur in the lichen transplanted material. A contamination index (C.I.) was calculated from the amounts of sulphur, chlorophyll-α, phaeophytin-α, and MDA.

KEY WORDS: Lichen, biomonitoring, sulphur, chlorophyll, soluble proteins, MDA, heavy metals

INTRODUCTION

Lichens are known to be sensitive to many types of pollution and are good biological monitors of air quality. Several biological variables have been used to assess pollution damage to lichens, including respiration (Baddeley *et al.*, 1972), photosynthesis (Showman, 1972; Puckett *et al.*, 1973; Ronen *et al.*, 1984) and chlorophyll degradation (Ronen and Galun, 1984; Garty *et al.*, 1985; Kardish *et al.*, 1987; Garty *et al.*, 1988).

The effects of air pollution have been recorded in lichens in their natural habitat, or in lichens transplanted from clean to polluted areas (Brodo, 1961; Ferry and Coppins, 1979; Ronen *et al.*, 1984; Garty *et al.*, 1985, 1988). Sulphur dioxide is a very common pollutant and is reported to be especially harmful. Probably the absence of lichens in cities and industrialized areas is due to an interaction between sulphur dioxide and other factors associated with urban environments such as other gaseous and airborne pollutants, changes in air temperature and humidity, and above all, vegetative cover (Pearson, 1973). The method most often used to study lipid peroxidation is the measurement of thiobarbituric acid-reactive substances such as malondialdehyde (MDA), as lipid peroxides reacts with thiobarbituric acid (Kappus, 1985).

To date, MDA has not been used in field studies as an indicator to assess pollution in lichens. In this study we examine the possibility of using MDA concentration to monitor the state of lichens exposed to air pollution. We have related the MDA level to other variables, such as soluble protein concentration, chlorophyll degradation and the levels of six heavy metals and sulphur detected in the lichen, *Punctelia subrudecta* (Nyl.) Krog, transplanted to urban sites in Córdoba, Argentine.

In the present study we use data on the chemical content of lichens for two purposes: a) to obtain information about the ability of *Punctelia subrudecta* to indicate air pollution, and b) to obtain information about relative air quality. In Córdoba city, because air pollution data do not exist, a system of bio-monitoring was employed as a first step to obtaining pollution density patterns. Such information is assumed to be provided by making comparisons between the pollutants detected in the same lichen species growing in rural sites and in sites considered urban; the air in the latter is expected to be polluted by dense traffic and industry.

MATERIALS AND METHODS

Biological material

Thalli of the lichen, *Punctelia subrudecta* (Nyl.) Krog., were used as a model organism to monitor air pollution. The lichen was collected from a site near La Calera, North West Córdoba, Argentina, a place known to be "clean" with respect to air pollution.

Collecting procedure

The lichen colonies were first rinsed with distilled water and then removed from the substrate by scraping with a scalpel. A part of this freshly picked material was separated and subjected to the same chemical analysis as carried out on the transplanted material, so as to obtain a base level for the sampling.

Lichen bags were prepared by weighing out approximately 6 g lichen (wet weight), and packing this loosely in a fine nylon hair-net. These nets produce an insignificant area of exposed nylon, which, while supporting the lichen, has minimal interference with the entrapment and collection properties of lichen.

Study area and lichen transplantations

In December 1990, lichen bags were transplanted to ten different monitoring stations (Figure 1) on trees at a height of 3 m for each of the sites in the region of study (Table I). Lichen bags were harvested in March 1991, but were not recovered from 3 sites. After the lichens had been exposed and before chemical analysis, each sample (6 g) was shredded in order to achieve homogeneity. This produced more representative samples for each analysis. As small quantities are used, this minimizes the error due to the fact that the initial sample is made up of many individuals, probably of different ages and physiological condition.

Heavy metals

The thalli were rinsed with distilled water, and then dried, wet ashed, and prepared for heavy metal determination, based on the method of Shimwell and Laurie (1972). Digestion was followed by washing in a 1:4:10 mixture of concentrated acids ($HClO_4$, H_2SO_4 and HNO_3). After complete evaporation of the acids, the resulting white residue was transferred to concentrated hydrochloric acid as the medium for analysis.

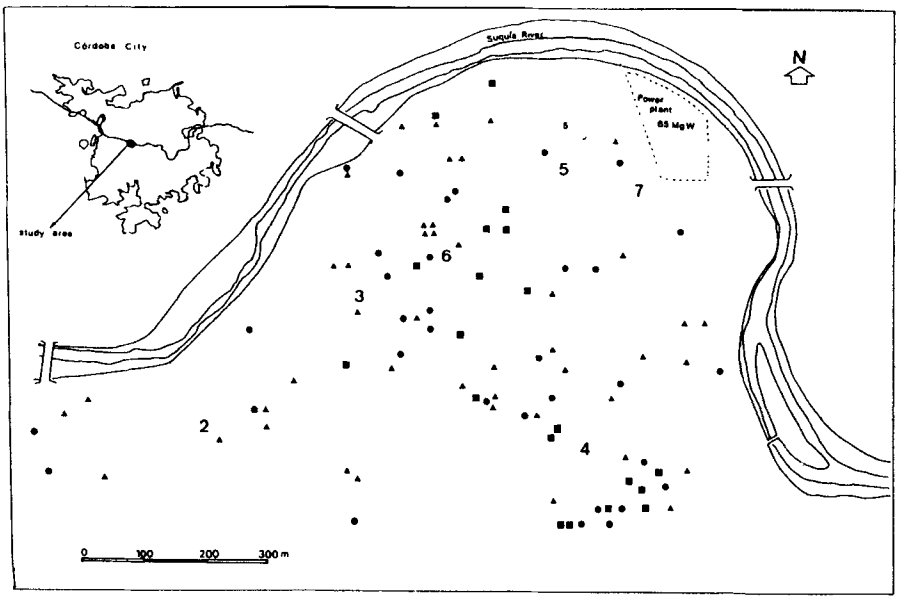

Figure 1 The sites of *P. subrudecta* transplantation (2 to 7) in Córdoba, Argentina. Factory sites: ○ particle emissions (cement works, carpentries, sheet metal works), □ chemical pollutants emission of (body shops, petrol stations, industrial cleaning), △ emission of chemical pollutants and particles (metallurgical workshops, car tyre remoulding businesses)

Table 1 Transplantation sites

Site	*Automobile Activity*	*Industrial Activity*	*Characteristics*
2	Low	Low	High humidity
3	High	High	Mainly metallurgical industries and car tyre remoulding businesses
4	High	Moderate	Mainly paint workshops, sheet metal workshops, petrol stations and industrial cleaning.
5	Low	Low	
6	Low	Moderate	Mainly small metallurgical workshops.
7	Moderate	High	Power station powered by fuel-oil. Capacity: 65 MW.

The content of six metals (Pb, Zn, Cu, Al, Fe and Mn) was then determined for each sample using a Jarrell-Ash 82-500 MVAA atomic absorption spectrophotometer employing flame emission, with comparison with standard spectrosol metal solutions, for atomic absorption analysis (BCS and NBS, England).

Chlorophyll measurements

The plant material was freeze-dried; there were four replicates. After the lichen materials had been frozen and subsequently ground into powder in a Polytron grinder, chlorophylls and phaeophytins were extracted with 80% reagent grade acetone. To avoid phaeophytinization of chlorophyll in lichens containing acidic lichen substances, the following procedure was adopted. The lichen was finely ground with excess sodium carbonate (150 mg carbonate to 300 mg lichen) and pigments extracted in 80% acetone for 1 h in the dark (Rao and Leblanc, 1966).

The acetone extracts were filtered to remove solid debris. Afterwards, oxalic acid-acetone 80% v/v saturated was added to the clear chlorophyll extract (0.3 ml oxalic acid solution and 9.7 ml chlorophyll extract) in order to induce phaeophytin formation.

Absorption of chlorophylls and phaeophytins, and the phaeophytins alone (after addition of oxalic acid), was measured with a Bausch & Lomb Spectronic 21 spectrophotometer. The concentrations of the chlorophyll-α and phaeophytin-α were determined according to the equations established by Vernon (1960).

Protein determination

After the lichen material had been frozen and subsequently reduced to a powder, soluble proteins were extracted with sodium phosphate buffer 0.1 M; pH 7.0. Protein determination was carried out according to Kalckar (1947). Absorption at 280 nm and 260 nm was recorded in a Bausch & Lomb Spectronic 21 Spectrophotometer.

Sulphur content

Absorption of sulphur compounds has often been measured by an increase in sulphur content using a turbidimetric method. Lichens were oven-dried at 55 °C and pulverized by a mill. About 0.5 g of the lichen powder was measured accurately. Magnesium nitrate saturated solution was added and the solution heated. After the sample was calcined at 500°C, it was suspended in 6 N hydrochloric acid and filtered at low pressure. The amount of sulphate present in solution was determined by the acidic suspension method with barium chloride (Toennies and Bakay, 1953).

Peroxidation product estimation

Malondialdehyde (MDA) was measured by a colorimetric method (Heath and Parker, 1968). A sample (50 mg) of *P. subrudecta* was homogenized in 2.5 ml of distilled water. An equal volume of 0.5% TBA (2- thiobarbituric acid) in 20% trichloroacetic acid solution was added and the sample incubated at 95 °C for 30 min. The reaction was stopped by putting the reaction tubes in an ice bucket. The samples were then centrifuged at 10,000 g for 30 min. The supernatant was removed and read at 532 nm, and the value for nonspecific absorption at 600 nm was read and subtracted from this. The amount of MDA present was calculated from the extinction coefficient of 155 mM (Kosugi *et al.*, 1989).

Table II Heavy metal content (ppm on dry weight basis) of freshly picked *P. subrudecta* (F) at La Calera and in material transplanted (T) to six biomonitoring sites. [a] Arithmetic mean values. [b] Standard deviation.

Site	*Pb* *ppm* *Mean*[a] *S.D*[b]	*Fe* *ppm* *Mean S.D*	*Zn* *ppm* *Mean S.D*	*Al* *ppm* *Mean S.D*	*Cu* *ppm* *Mean S.D*	*Mn* *ppm* *Mean S.D*
(F)	29.0±5.8	1031±200.1	38.5±7.9	1193±236.3	22.0±16.4	52.5±5.1
2(T)	37.4±7.8	1372±262.2	51.4±11.5	2482±368.6	26.7±26.2	69.4±13.2
3(T)	72.0±14.1	3729±482.3	88.3±17.4	3917±547.7	49.7±7.2	110.4±21.3
4(T)	62.5±9.3	3921±167.2	85.2±13.2	4000±608.3	62.5±12.3	102.3±9.2
5(T)	38.5±7.2	1222±323.5	46.3±7.6	2560±376.8	23.4±5.1	60.6±10.4
6(T)	38.6±6.1	1851±275.5	54.6±8.2	3525±528.6	27.5±6.3	66.1±12.2
7(T)	67.4±9.8	1774±256.9	56.4±10.3	3402±630.8	28.1±5.8	78.6±14.1

RESULTS

The metal content of freshly picked *P. subrudecta* from La Calera and lichen bags recovered after transplant at six sites (2-7) after brief exposure (December 1990- March 1991) are presented in Table II.

The highest total metal content was found in the lichens transplanted to site 4 and the lowest was found in freshly picked material. The highest amounts of zinc, copper, iron and aluminium were detected in the lichen exposed at sites 3 and 4. Lichens transplanted to sites 3, 4 and 7 accumulated the highest amounts of lead.

The sulphur content of lichen transplanted to site 7 (near power plant) was higher than that of lichens transplanted to most other sites (Table III).

An increase in the MDA content, sulphur and insoluble protein content was observed in lichens transplanted to all six sites in comparison with freshly picked material (F).

Among the six sites to which lichens were transplanted, all exhibited a significant decrease in chlorophyll α (Table IV) with respect to freshly picked *P. subrudecta* (F),

Regression equations for relationships between measured variables are given in Table V.

Table III Sulphur content, soluble protein concentration and MDA concentration of freshly picked *P. subrudecta* (F) at La Calera and in material transplanted (T) to six biomonitoring sites.[a] Arithmetic mean values. [b] Standard deviation.

Site	*S content* *(mg/g DW)* *Mean*[a] *S.D.*[b]	*Soluble protein* *(mg/g DW)* *Mean S.D.*	*MDA* *(nmoles/g DW)* *Mean S.D.*	*DW/WW* *g/g*
(F)	1.75±0.001	48.65±0.01	30.0±0.02	0.897
2(T)	3.06±0.004	102.20±0.009	223.0±0.39	0.936
3(T)	2.86±0.006	76.37±0.002	271.4±0.97	0.905
4(T)	2.84±0.005	66.06±0.003	225.2±0.65	0.879
5(T)	3.10±0.002	63.63±0.001	190.3±0.42	0.908
6(T)	3.19±0.003	52.16±0.002	202.1±0.36	0.908
7(T)	3.45±0.004	105.10±0.009	246.3±0.86	0.890

Table IV Chlorophyll α concentration, Phaeophytin α concentration and chl.α/phaeoph.α ratio of freshly picked *P. subrudecta* (F) at La Calera and in material transplanted (T) to six biomonitoring sites. [a] Arithemetic mean values. [b] standard deviation.

Site	*Chl.α* *mg/g D.W.* *Mean*[a] *S.D.*[b]	*Phaeoph.α* *mg/g D.W* *Mean S.D.*	*Phaeoph.α/Chl.α*
(F)	0.672±0.080	0.564±0.048	0.84
2(T)	0.316±0.024	0.477±0.027	1.51
3(T)	0.272±0.036	0.351±0.019	1.29
4(T)	0.351±0.011	0.445±0.041	1.27
5(T)	0.359±0.021	0.449±0.052	1.25
6(T)	0.296±0.032	0.356±0.041	1.20
7(T)	0.406±0.046	0.470±0.053	1.66

Table V Regression parameters for relationships of *Punctelia subrudecta* from biomonitoring sites (2–7) and freshly picked (F).

Relationships	*Regression equation*	R^2	*Sample size*
Chlorophyll α-Al	y = 0.0001x–0.7	0.63	7
Chlorophyll α-S	y = 0.198x–0.96	0.64	7
MDA-Phaeophytinization	y = 0.002x + 0.82	0.61	7
S-Phaeophytinization	y = 1.86x + 0.63	0.46	7
Pb-MDA	y = 3.36x + 32.6	0.54	7
Al-MDA	y = 0.068x + 7.30	0.75	7
S-MDA	y = 123.0x + 157.0	0.72	7
C.I-Protein	y = 1.78x + 37.89	0.44	7
C.I-Pb	y = 1.40x + 21.48	0.46	7
C.I-Al	y = 96.5x + 1089.5	0.66	7

Contamination Index

A contamination index, C.I., was determined by using the formula:

$$C.I. = [(P.\alpha/C.\alpha) + (S_T/S_F)] * (MDA_T/MDA_F)$$

where, P.α is the phaeophytin-α of lichen in mg g^{-1} dry weight, C.α is the chlorophyll-α of lichen in mg g^{-1} dry weight, S_T is the sulphur content of lichen in each transplanted

Table VI Contamination Index (C.I.) in freshly picked *P. subrudecta* at la Calera (F) and in material transplanted (T) to six biomonitoring sites.

Site	*C.I*
(F)	1.84
2 (T)	24.22
3 (T)	26.45
4 (T)	21.64
5 (T)	19.16
6 (T)	20.36
7 (T)	25.71

lichen sample in mg g^{-1} dry weight, S_F is the sulphur content of lichen freshly picked, MDA_T is the MDA in each transplanted lichen in n mol g^{-1} dry weight, and MDA_F is the MDA content in lichen freshly picked in n mol g^{-1} dry weight. The results of C.I. are presented in the Table VI.

The highest value was found for lichens transplanted to site 3 and the lowest for freshly picked *P. subtrudecta* (F).

DISCUSSION

In the present study, the MDA concentrations were related to the sulphur content ($R^2 = 0.72$) and the ratio of phaeophytin α/chlorophyll α (phaeophytinization) ($R^2 = 0.61$).

The increase in MDA in the lichen transplanted to sites 2–7 (Table III) during the December 1990–March 1991 period suggests that MDA concentration is sensitive to certain air pollutants.

We have found less correlation between chlorophyll degradation expressed as the phaeophytinization ratio and sulphur content ($R^2 = 0.46$) than between MDA and the sulphur content ($R^2 = 0.72$) in transplanted *P. subrudecta* (Table V).

MDA and phaeophytinization in *P. subrudecta* are effected in field conditions by the presence of sulphur ($R^2 = 0.72$ and $R^2 = 0.46$ respectively) as well as possibly by other factors and ambient relative humidity. We have found a direct relation between MDA and aluminium ($R^2 = 0.75$), MDA and lead ($R^2 = 0.54$) and MDA and sulphur ($R^2 = 0.72$) in a transplantation experiment. However this was not significant between phaeophytinization and aluminium ($R^2 = 0.24$) or lead ($R^2 = 0.06$) (data not shown). The main source of lead emitted into the air in the whole study area is leaded gasoline, which is still in use in Argentine; the main sources of aluminium are small metallurgic industries. We found an inverse relation between chlorophyll α and aluminium ($R^2 = 0.63$) and with sulphur ($R^2 = 0.64$) but not for chlorophyll α and lead ($R^2 = 0.20$) (data not shown).

It seems that degradation of chlorophyll in lichens takes a much longer time than other physiological damage during the exposure of thalli in polluted conditions. Such damage may effect the integrity of the cell membranes in different ways, such as penetration of toxic ions and compounds into the cell cytoplasm, leakage of essential ions and destruction of thyllakoids (Garty *et al.*, 1988).

The present study showed that in most sites with a high level of vehicular traffic (sites 3 and 4), the level of metal content was high. According to Christensen and Guinn (1979), zinc particles may be derived from industrial sources, while the abrasion of motor vehicle tyres may be a second source of emission. We may conclude that most of the heavy metals detected in the thalli of the transplanted lichens are derived from anthropogenic activity at urban sites. We assumed that the sulphur content in the lichen would provide a good estimate of atmospheric sulphur dioxide concentrations at urban sites (Garty *et al.*, 1988). This assumption is based on a series of experiments that demonstrated such a relation in plants (Gilbert, 1969; Takala *et al.*, 1985).

The protein concentration was not significantly related to the other variable analyzed, the C.I. R^2 being 0.44 (Table V).

The contamination index (C.I) determined from the chemical measurements in *P. subrudcta* indicate various atmospheric pollution levels in comparison with the "clean"

site (La Calera). The major sources of pollutants in the city area are automobile exhausts and small industries (except site 7). In ascending order of pollution: F (initial, freshly picked), 5 (low automobile pollution and low industrial pollution), 6 (low automobile pollution and moderate industrial pollution), 4 (high automobile pollution and moderate industrial pollution), 2 (low industrial pollution, low automobile pollution but high humidity), 7 (moderate automobile activity and high industrial pollution - power plant) and 3 (high automobile pollution and industrial pollution).

Site 2, even though it has "low" pollution, has a high humidity. According to Rao and Le Blanc (1966), high levels of humidity promotes the action of contaminants on the majority of lichen species. This is probably reflected in the high value of the C.I. for this site. Although site 2 is far from the Power Plant and has low traffic, it is localized within the urban area. So, the relatively high contamination index (C.I.) for this site indicates a diffusional phenomenon potentiated by the humidity.

There was a direct relationship between C.I. and the heavy metals in the case of aluminium ($R^2 = 0.66$) and lead ($R^2 = 0.46$) (Table V).

Finally, we recommend the continued use of *P. subrudecta* transplants as biomonitors at urban sites, as well as near the electricity generating power plant, and we also recommend further study of the effect of these pollutants on MDA, chlorophyll degradation and soluble protein content, both in the field and in laboratory conditions.

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